Sampling, Modelling and Prediction for Freshwater Species Across River Ecosystems:

An example with the Freshwater Pearl Mussel

Margaritifera margaritifera (L., 1758)

SABELA LOIS LUGILDE

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Abstract

The general objectives of this thesis are to develop a complete process of sampling, modelling and prediction for freshwater species focused on biotic interactions between species within rivers across a regional landscape. To address this objective the endangered freshwater mussel (*M. margaritifera*) that must pass its larval stage attached to a host fish is used for gaining biological and ecological information relevant to its conservation.

Resumo

O obxectivo xeral desta tese é desenvolver un proceso completo de mostraxe, modelización e predición para especies fluviais, centrándose nas interaccións bióticas entre especies dentro de redes fluviais a escala rexional. Para abordar este obxectivo, o mexillón de auga doce en perigo de extinción (*M. margaritifera*), que ten unha etapa larval parasita nun peixe hospede, utilízase coa fin de obter información biolóxica e ecolóxica relevante para súa conservación.

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TESE DE DOUTORAMENTO

Sampling, Modelling and Prediction for Freshwater Species Across River Ecosystems:

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Margaritifera margaritifera (L.,1758)

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Programa de doutoramento “Medio Ambiente e Recursos Naturais”

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An example with the Freshwater Pearl Mussel *Margaritifera margaritifera* (L.,1758)

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AUTORIZACIÓN DOS DIRECTORES DA TESE

Dra. Mª Paz Ondina Navarret e Dr. Adolfo Outeiro Rodríguez profesores titulares do Departamento de Zooloxía e Antropoloxía Física da Universidade de Santiago de Compostela, como Directores da Tese de Doutoramento titulada "Sampling, Modelling and Prediction for Freshwater Species Across River Ecosystems: An example with the Freshwater Pearl Mussel *Margaritifera margaritifera* (L.,1758)" presentada por Dna. Sabela Lois Lugilde, alumna do Programa de Doutoramento Medio Ambiente e Recuros Naturais,

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“Non hai tolo sen idea”

“The most difficult environment to change is the one around you”

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III. Aknowledgments

This thesis is aimed at describing a whole process of dealing with rivers, mussels fish and humans and trying to learn how to be a PhD in the land of a thousand rivers…my God how many things…and right now it feels like I can not believe that I am writing the acknowledgements of my thesis, it seems like writing on the head stone of a Grave!!!… I am describing how maybe every PhD student feels in this moment…but yes, it is over, I am not dreaming or in a nightmare, it's real. So let's write the epitaph of this thesis, and congratulate all the people that for me were a pleasure to meet during this processes.

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Finally, I am happy to reach this stage in which I have a better understanding of complex ecosystems such as rivers and that I have done it in my homeland. Although it was a complex process, I am lucky to say that I learned what doing research means. In this context I want to pay tribute to all Spanish researchers that fight here to do research, especially to the ones that are forced to leave the country to work elsewhere, or to abandon research altogether. I hope in short that all our painful efforts will change the research culture of this country.

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IV. Abstract

English

There is a general need to provide integrative studies to understand, retain and simplify the information contained in spatial patterns of biodiversity in complex ecosystems such as rivers. The general objectives of this thesis are to implement a complete process of sampling, modelling and predicting for freshwater species across and within river networks focused on biotic interactions. To address this objective the endangered freshwater mussel *Margaritifera margaritifera* (L., 1758) that must pass its larval stage attached to a host fish, is used to gain biological and ecological knowledge to manage its conservation.

This study was carried out in river networks within the boundaries of the autonomous region of Galicia (Northwest Spain) in Europe. It provides the first extensive study about modelling and predicting to understand distribution and abundance patterns of *M. margaritifera* focused on biotic interactions between the species and its hosts. The ecological and biological understanding gained in this work will serve as a basis to develop conservation strategies for *M. margaritifera* in the Iberian Peninsula and in other parts of its whole distribution range. Furthermore, the thesis is the first on *M. margaritifera* in the Iberian Peninsula and it provides the first data of its distribution and conservation status in Galicia.

The sampling design was based on a combination of qualitative and quantitative methods to identify the current distribution of the species, estimate population densities and search for small-sized individuals, which may be indicative of recruitment. The results showed that Galicia has the major number of *M. margaritifera* populations in the Iberian Peninsula and using age profiles recuritment was documented in four rivers.This assessment was an effective technique to cover large areas providing the necessary data for addressing modelling and prediction. For modelling mussel distribution and abundance, 16 predictors belonging to four categories (geologic, climatic, landform, and biotic factors) were designed to use as covariates for different modelling techniques. Model results indicated that biotic interactions influenced species

distribution and abundance at a regional scale. Thus, spatial patterns due to biotic interactions can be manifested at a broad-scale between *M. margaritifera* and its hosts.

The machine learning technique (MaxEnt), used for distribution modelling, easily handled the landscape complexity of Galicia to predict probability of species presence and it helped to discern the effect of biotic interactions when predicting species presence. Path analysis and geostatistical mixed models for stream networks were used to model mussel abundance data and the difference in variance explained indicates the necessity of accounting for different sources of spatial autocorrelation when modelling river ecosystems. Analysis of subsets of data approximated the influence of dams on availability of migratory host fish, indicating the importance of migratory fish controlling mussel abundance. Model results indicated that biotic interactions cause spatial autocorrelation in abundance. The spatial autocorrelation scale can be used as a practical biogeographical scale for assessment, planning, habitat management, and administration of multi-species conservation programs. Abundance predictions and assessment of population structure showed areas where biotic interactions between mussels and fish are still occurring, helping to identify conservation strategies for preserving biotic interactions between mussels and host fish.

In general, accounting for biotic interactions when modelling is a necessary step to understand ecology and address conservation of interacting species. In order to address modelling and predicting species when they occur in ecological networks such as rivers the directional topology of the network should be considered. Although the techniques and processes described are applied to the endangered freshwater pearl mussel *M. margaritifera* in different rivers in Galicia, the novel integrative process and the analytical techniques described in this study may be applied to any freshwater riverine species. In that sense, research to address different hypotheses and objectives can be carried out by applying the processes described in this thesis for other species in different freshwater riverine environments of other geographical regions.

Galego

Existe unha necesidade de proporcionar estudos enfocados a comprender, reter e simplificar a información contida nos patróns espaciais da biodiversidade en ecosistemas complexos tales como os ríos. O obxectivo xeral desta tese é desenvolver un procedemento completo de mostraxe, modelización e predición para especies fluviais, centrándose especialmente nas interaccións bióticas entre especies. Para abordar este obxectivo utilizarase o mexillón de agua doce en perigo de extinción (*Margaritifera margaritifera* (L., 1758), que ten unha etapa larval parasita nun peixe hóspede, coa fin de obter información biolóxica e ecolóxica para xestionar a súa conservación.

Este estudo, realizado nas redes fluviais dentro dos límites administrativos da rexión autónoma de Galicia (noroeste de España), proporciona o primeiro estudo extenso sobre modelización da distribución e abundancia de *M. margaritifera* centrado nas interaccións bióticas cos seus peixes hóspedes. Os avances e os novos coñecementos obtidos deste traballo servirán de base para o desenvolvemento de estratexias de conservación de *M. margaritifera* tanto na Península Ibérica coma noutras partes da súa área de distribución. Por outra banda, esta é a primeira tese sobre *M. margaritifera* na Península Ibérica onde se proporcionan os primeiros datos sobre a súa distribución e o seu estado de conservación en Galicia.

O método de mostraxe utilizado con *M. margaritifera*, baséase nunha combinación de métodos cualitativos e cuantitativos, o que permitiu coñecer a súa distribución actual, estimar a densidade de poboación así como detectar individuos de pequeno tamaño que poderían ser indicativos de recrutamento poboacional. Os resultados da mostraxe indican que Galicia conta co maior número de poboacións *M. margaritifera* na Península Ibérica e que mediante o uso de perfis de idade detectouse recrutamento en catro ríos. Esta técnica de mostraxe resultou eficaz para cubrir grandes áreas e proporcionou os datos necesarios para aplicar métodos de modelización e predición. Para a modelización da distribución e da abundancia, seleccionouse un conxunto de 16 preditores pertencentes a catro categorías (xeoloxía, clima, relevo e factores bióticos) para utilizar como covariables nas diferentes técnicas de modelado.

Estes modelos indicaron que as interaccións bióticas inflúen na distribución e abundancia de *M. margaritifera* a escala rexional. Polo tanto, os patróns espaciais debido ás interaccións bióticas poden manifestarse a ampla escala entre *M. margaritifera* e os seus hóspedes.

O algoritmo de máxima entropía de aprendizaxe automático (MaxEnt) utilizado para o modelado da distribución xestionou, de forma eficaz, a complexidade predicindo a probabilidade da presenza de *M. margaritifera* e axudando a discernir o efecto das interaccións bióticas. Cos dos datos de abundancia utilizáronse a técnica de "path analyse" e os modelos xeoestatísticos mixtos para redes fluviais. As diferenzas na varianza explicada destes modelos indicaron a necesidade de ter en conta as diferentes fontes de autocorrelación espacial nos ecosistemas fluviais. A análise de subconxuntos de datos aproximou a influencia dos encoros na dispoñibilidade de hóspedes migratorios indicando a importancia dos peixes migratorios no control da abundancia de *M. margaritifera*. Os resultados dos modelos indican que as interaccións bióticas son un compoñente de autocorrelación espacial da abundancia. A escala da autocorrelación espacial podería ser utilizada como una escala bioxeográfica práctica para a avaliación, planificación, e xestión do hábitat así coma para a administración dos programas de conservación enfocados a conservación das interaccións bióticas. As predicións da abundancia e a avaliación da estrutura das poboacións mostraron áreas nas que as interaccións bióticas entre *M. margaritifera* e os seus hóspedes seguen a producirse. Esta metodoloxía axudou identificar estratexias para a conservación das interaccións bióticas entre mexillóns e os seus hóspedes.

En xeral, este traballo mostrou a necesidade de incluír as interaccións bióticas nos modelos como paso esencial para entender a ecoloxía e abordar a conservación das especies que interactúan. Alén diso, para desenvolveren procesos de modelización e predición das especies que habitan nas redes ecolóxicas, tales como ríos, debe terse en conta a topoloxía direccional. As técnicas e os procedementos descritos neste traballo aplícaronse ao mexillón de auga doce (*M. margaritifera*) en Galicia. No entanto, este novidoso proceso xunto coas as técnicas de análise pódense aplicar a calquera especie

fluvial. Neste sentido, mediante a aplicación dos procesos descritos, as investigacións para estudar distintas hipóteses e obxectivos poderían levarse a cabo para outras especies en diferentes ecosistemas fluviais doutras rexións xeográficas.

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1. INTRODUCTION

”Cando o río soa é que auga leva”

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1. INTRODUCTION

1.1. Background and Statement of the Problem

Freshwater is an essential resource for life and it was recognized to be a critical factor for meeting all the objectives of the eight Millennium Development Goals by the United Nations for 2015. Freshwater ecosystems and their biodiversity are being recognized to be a valuable resource from the economic, cultural, aesthetical, scientific and educational points of view, and their conservation is critical to the interest of human beings and nations worldwide. However, compared with other ecosystems freshwaters and their biota are experiencing higher declines in biodiversity (Dudgeon *et al.,* 2006). Thus, there is an urgent need to provide evidence-based knowledge (Sutherland *et al.,* 2004) to understand biodiversity patterns in freshwaters and manage their conservation.

Among freshwaters, riverine ecosystems are the most complex environments. They are water networks embedded in the landscape where the drainage basin or watershed is the terrestrial unit area that delimits hydrological processes on a river. This is why from a landscape perspective drainage basins are sometimes considered as biogeographical islands (Sepkoski & Rex, 1974; Simberloff, 1974; Minshall *et al.,* 1983; Hugueny, 1989). Thus, in each river the specific landscape characteristics of the watershed will influence stream ecosystem structure and function from a regional scale (drainage basin) to a local scale (microhabitat) (Roth *et al.,* 1996; Allan *et al.,* 1997). The spatial characteristics of rivers within drainage basins creates an interconnected series of biotopes and environmental gradients where fluxes of matter and energy can occur through interactive pathways across four different dimensions longitudinal, vertical, lateral and temporal (Ward, 1998). Moreover, the human impacts to rivers worldwide add another source of spatial heterogeneity. These facts make it a challenging multidisciplinary task to understand the physical, chemical, biological and anthropogenic processes that are occurring on river ecosystems of our planet. To address issues in a holistic way, a global perspective of the study of the broad-scale processes and patterns governing fluvial systems was defined under the concept of riverscape or riverine

landscape (Schlosser 1991; Fausch *et al.,* 2002; Ward *et al.,* 2002; Allan, 2004). This term served as a conceptual landscape scale for the study of biodiversity patterns and processes in riverine ecosystems; however, with the development of new analytical techniques in landscape ecology and spatial analysis, this concept continues to evolve (Allan, 2004).

It is widely recognized that conservation of biodiversity is facilitated by maintaining population densities and distributions of strongly interactive species (Soulé *et al.*, 2005), but there is widespread controversy about the spatial scale at which biotic interactions are identifiable (Araújo & Rozenfeld, 2014). The Eltonian noise hypothesis (Soberón & Nakamura, 2009) posits that biotic interactions may have measurable effects at small scales (Huston, 1999; Pearson & Dawson, 2003) but not at broader scales. Some works have challenged this idea (Araújo & Luoto, 2007; Heikkinen *et al*., 2007; Meier *et al.*, 2010; Kissling *et al.*, 2012; Araújo & Rozenfeld, 2014; de Araújo *et al.*, 2014) and others have called for more experimental examples and tools to support theoretical models (Wisz *et al.*, 2013). Recent simulations have suggested that the identifiability of biotic interactions across spatial scales may depend on the type of interaction (Araújo & Rozenfeld, 2014), with net positive interactions such as mutualism or commensalism likely to be manifested across scales. Independently of the spatial scale and the type of interaction, however, studies of biotic interactions in species distribution models are lacking for spatial networks such as rivers. In this thesis, distribution and abundance data for the freshwater pearl mussel *Margaritifera margaritifera* (Linnaeus, 1758) are presented and analysed.

Freshwater mussels are bivalves of order Unionoida commonly called naiads. There are estimated to be up to 840 species spread globally in freshwaters of all continents except Antarctica (Bogan, 2008; Graf & Cummings, 2007). Their life history characteristics provide a good target species for the study of river ecosystem processes that govern biodiversity patterns. They are benthic filter feeders and their high biomass implies they carry on important roles in particle processing, nutrient cycling, and sediment mixing which provides important ecosystem services in rivers (Spooner &

Vaughn, 2008; Vaughn *et al.,* 2008). Thus, their conservation has important benefits for managing quality of freshwater and maintaining critical ecosystem functions. The dependence of mussels on a mobile host fish for larval survival and dispersal makes them key species for gaining understanding about riverine biotic interactions, organism dispersal and river connectivity. However, they are probably the most endangered groups of animals (Bogan, 1993; Williams *et al.,* 1993; 2008; Strayer *et al.,* 2004) and there is a need for broad scale comprehensive studies to understand which factors are controlling mussel populations (Strayer, 2008) and to design integrative management actions for different areas.

M. margaritifera is the most widespread freshwater mussel species inhabiting rivers of the Holartic ecozone in North America and Europe. It uses different salmonid hosts across its distribution range (Taeubert *et al*., 2010) and it is considered a key species for the conservation of aquatic ecosystems (Geist, 2010). Although *M. margaritifera* is threatened with extinction throughout its geographic range, few studies have focused on modelling its distribution (Wilson & Roberts., 2011; Wilson *et al*., 2011a,b; Prié *et al.,* 2014) prior studies have not modelled its distribution and abundance patterns in the context of biotic interactions with its hosts. In this context, the northwest quadrant of the Iberian Peninsula is in the southern range limit of the European distribution of the species. Within the Iberian Peninsula the region of Galicia, was said to be a region where *M. margaritifera* was historically a common species in general terms (Macho, 1878) but no previous study had aimed at mapping its distribution in this area. Thus, the study of *M. margaritifera* in Galicia offers an opportunity to conduct an integrative study of a freshwater species to understand its current conservation status and gain ecological and biological understanding to manage its conservation.

This thesis describes a process of sampling, modelling and prediction for freshwater species across and within riverine ecosystems from a broad scale perspective (riverscape perspective). Thus, this work presents a case study based on the endangered freshwater pearl mussel *M. margaritifera* in Galicia. In the first part, a sampling process

for collecting extensive quantitative data about a species in its current riverine habitats is described. Then, in the framework of a bioclimatic envelope and biotic interactions, analyses of the current distribution of *M. margaritifera* in the study region are shown. Next, the thesis presents results of modelling abundance patterns when accounting for biotic interactions and river ecosystem characteristics. Finally, predictive modelling is used to identify conservation areas. For the future, the final chapter presents a draft proposal for values for microhabitat characteristics of the *M. margaritifera* populations in the study area.

1.2. Organism of Study: *Margaritifera margaritifera* (L.)

Life History and Ecology

The freshwater pearl mussel *M. margaritifera* is a bivalve mollusc species that belongs to the order Unionoida. This order represents the large mussel species that live in the different freshwater ecosystems all over the world; they are often called naiads. Naiads have a complex life strategy. These invertebrate species depend on vertebrate host species for completion of their life cycles. Thus, the parasite-host system is a primary life cycle characteristic of these large freshwater mussel unionid species (Figure 1.3A). The freshwater pearl mussel belongs to the family Margaritiferidae and in its adult stage is a benthic filter feeder (Figure 1.2F) that lives partly buried and distributed in patches across the riverbed (Figure 1.3B). In contrast, the larva (glochidium) is a parasite using a host fish for growth, metamorphosis and dispersal (Strayer, 2008).

The general morphological characters used for *M. margaritifera* shell identification are shown in Figures 1.1 and 1.2A-E. It has compressed elongated shell shape that can reach 15 cm of length. However, it is notable that these naiad species have a high degree of plasticity, making morphological characters sometimes not useful for their correct classification (Bauer, 2001) (Figure 1.2).

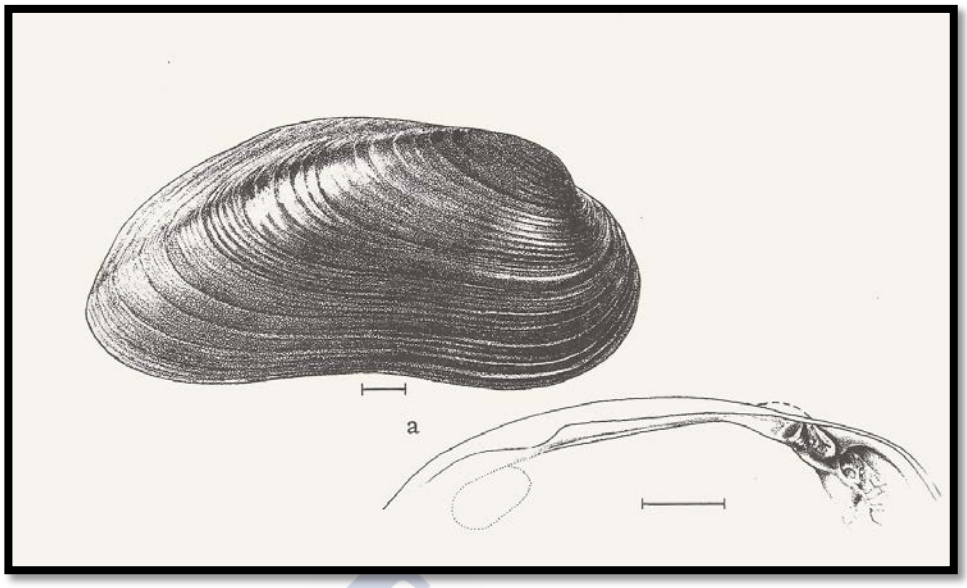


Figure 1.1.- General morphological characteristic for *M. margaritifera* shell identification (Burch, 1973).

The freshwater pearl mussel lives up to 200 years (Mutvei & Westermark, 2001) although its growth rates and longevity varies across its distribution range (Bauer, 1988, San Miguel *et al.*, 2004). It has high fecundity (Hastie & Young, 2003), and high lifetime fertility (Bauer, 1987). The species uses various salmonid hosts (Taeubert *et al.*, 2010) but in the region of this study (Galicia, NW Iberian Peninsula) the available native salmonids are Atlantic salmon (*Salmo salar*) and brown trout (*Salmo trutta*) (Hervella & Caballero, 1999). Although the glochidia were reported to encyst in fins in some hosts (Meyers & Milleman, 1977), its development generally occurs in the gills of the salmonids (Young & Williams, 1984). The glochidium requires up to 11 months to complete metamorphosis (Young & Williams, 1984) before it drops as a juvenile mussel from the host onto the river bed (Hastie & Young, 2003) (Figure 1.4).

It is remarkable that, among host-parasite relationships, *M. margaritifera* is considered unique, because the generation time of the parasite largely exceeds that of its hosts. It is said to be a stable life history strategy for at least 60 million years (Bauer, 2001). Moreover, from an ecological point of view, some authors have suggested that the hosts may benefit from mussel filter-feeding as well as the aquatic invertebrates associated with the microhabitat conditions created by mussels beds (Hastie & Cosgrove, 2001). Finally, during its juvenile post-parasitic stage they live buried in the

stream bed substratum for a period around 5 years, during which their survival to the adult stage will depend upon the oxygen available and its exchange between free-flowing water and interstitial water (Buddensiek *et al.*, 1993; Geist & Auerswald, 2007) (Figures 1.2,1.3 and 1.4).

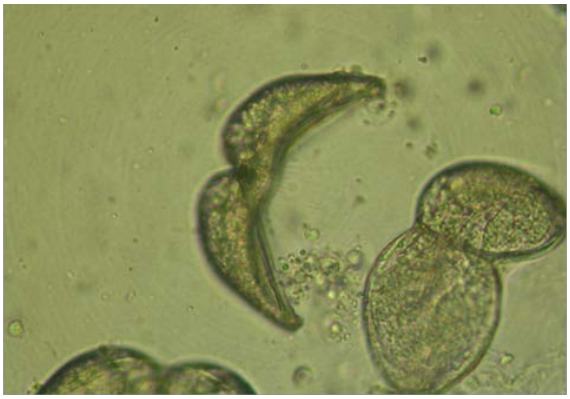
Figure 1.2.- The freshwater pearl mussel *M. margaritifera*. Shell. (A) Inside and (B) outside; (C) adult mussel and juvenile, (D) four-year old juvenile mussel; (E) several individuals of different sizes and (F) alive individuals filtering, detail of the siphons. Photos by Sabela Lois.

A

B

Figure 1.3.- (A) *M. margaritifera* life cycle; (B) picture of mussels filtering in aggregated in patches across the riverbed. Drawing and photo by S. Lois.

A



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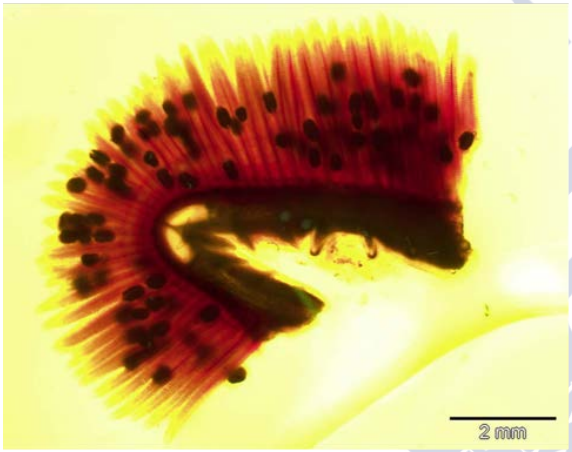

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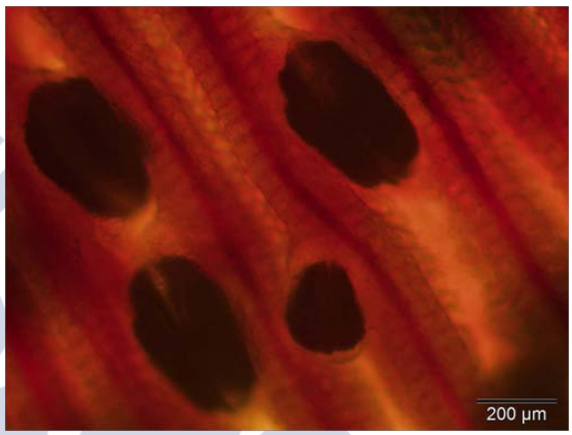

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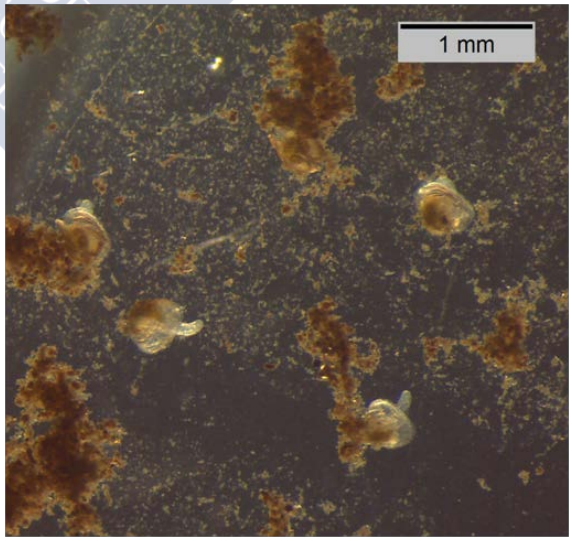

Figure 1.4.- Life stages of *M. margaritifera*. (A) Glochidium (photo by A. Outeiro), (B) detail of trout (*Salmo trutta*) gills with cysts, (C) Gill of trout with cysts (D), detail of cyst in the gills (photo by E. Taeubert), (E) and (F) post-parasitic stage of juvenile mussels of less 1 year old. Photos B, C, E and F by S .Lois.

Distribution and Conservation Status

The freshwater pearl mussel is distributed in fast flowing colder waters with high oxygen content in the rivers of the Holartic ecozone, from Arctic Russia (70º N) to the Iberian Peninsula (40º N) and it also occurs in North America (Young *et al.,* 2001a) (Figure 1.5 and 1.6). The species is considered endangered throughout its distribution range by the International Union for Conservation of Nature (IUCN, 2013), it is protected in the European Union under the Habitats Directive (Directive 92/43/EEC, Council of the European Communities 1992) and listed as endangered by the Autonomous Government of Galicia (Decreto 88/2007). The species has been called one of the most threatened freshwater bivalves in the world (Machordom *et al.,* 2003) and within Europe their populations have declined 90% during the last century (Araujo & Ramos, 2001).

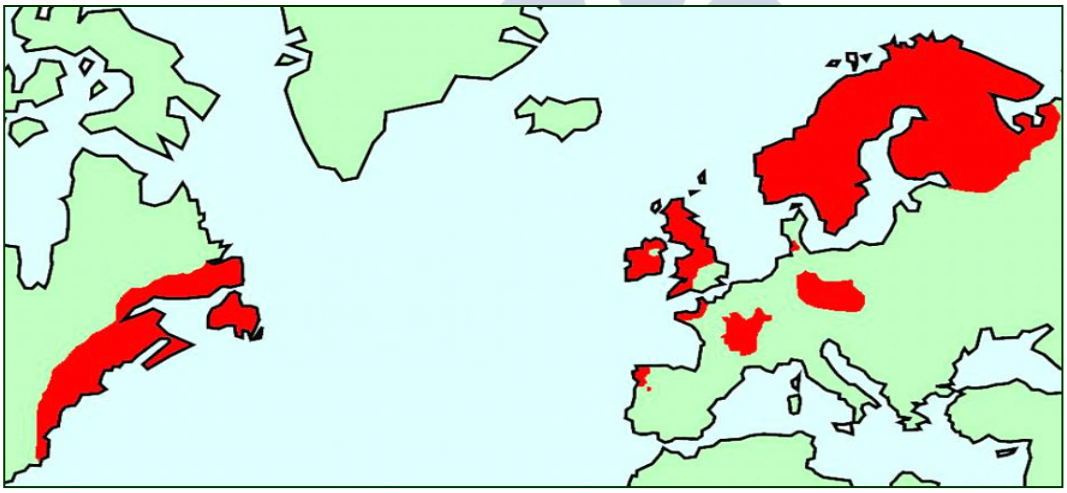

Figure 1.5.- Historical distribution of *M. margaritifera* (modified from Young *et al.*, 2001a)

From a conservation perspective, *M. margaritifera* was suggested to fulfil the criteria of indicator, flagship, keystone and umbrella species being an ideal target species for conservation of aquatic ecosystem functioning (for a review of conservation status and ecology around Europe see Geist, 2010). Thus, there are extensive efforts underway in Europe and North America to recover its populations through captive breeding and habitat management due to the lack of recruitment in most of its

populations (Gum *et al.,* 2011) (Figure 1.6). Despite this early and extensive investment in captive breeding, knowledge about the species' ecological requirements is still fragmented and limited (Geist, 2010; Varandas *et al.*, 2013). As the decline of various pearly mussels such us *M. margaritifera* is attributed to various factors such as habitat loss, pollution, climate change, dams, pearl fishing, and changes in salmonid stocks (Strayer *et al.,* 2004), it makes it a difficult task to discern the species ecological requirements. The Figure 1.7 shows several examples of freshwater mussel rivers in different countries in Europe.

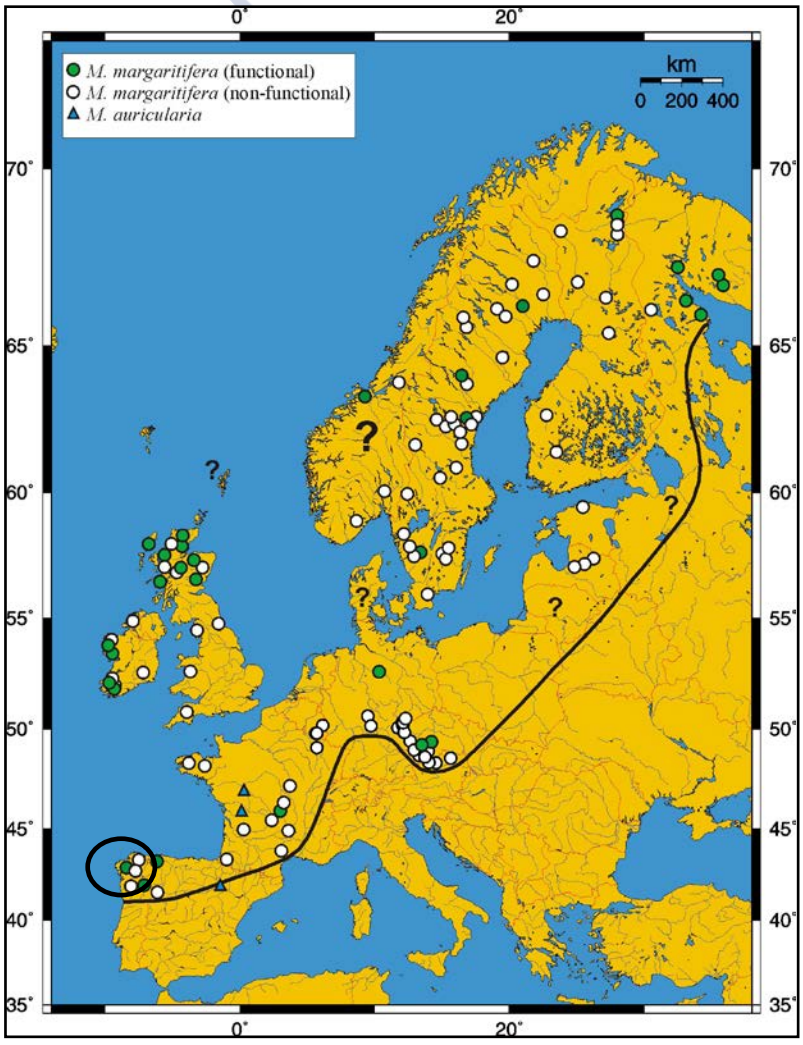

Figure 1.6.- *M. margaritifera* distribution in Europe; population status is functional (green dots) or non functional (white dots) (modified from Geist, 2010), black circle indicates the study area of this thesis.

A

B

C

D

Figure 1.7.- Different freshwater pearl mussel rivers in Europe (A) Ireland; (B) Germany; (C) Luxemburg and (D) Sweden. Photos by S. Lois.

One core issue that should be taken into account for *M. margaritifera* conservation is its dependence on the host fish. The successful development of the glochidia of *M. margaritifera* depends on availability of suitable host fish populations, Atlantic salmon and brown trout in this study (Figure 1.8). The Atlantic salmon formerly occurred in nearly all basins of the study area, but the species' contemporary distribution is highly contracted by development of hydropower dams on most large rivers in Galicia during the period 1955-1975 (Hervella & Caballero, 2002). The few remaining undammed rivers occupy small coastal drainages (World Commission on Dams, 2000). Stocks of Atlantic salmon continue to decline in Galicia and in much of the species' former European range (Garcia de Leaniz & Martínez, 1988; Parrish *et al.,* 1998; Hastie & Cosgrove, 2001). Brown trout is represented by two ecotypes in much of Europe, a resident form and a migratory one (anadromous) (Jonsson *et al.,* 2001; Marco-Rius *et al.,* 2012). The migratory trout have suffered declines similar to that observed for Atlantic salmon (Hastie & Cosgrove, 2001). Anadromous trout and Atlantic salmon spawn in the autumn to early winter (Behnke, 2002; Caballero *et al.,* 2006) and entry into rivers from the sea can be variable in different parts of the geographic range (Behnke, 2002).

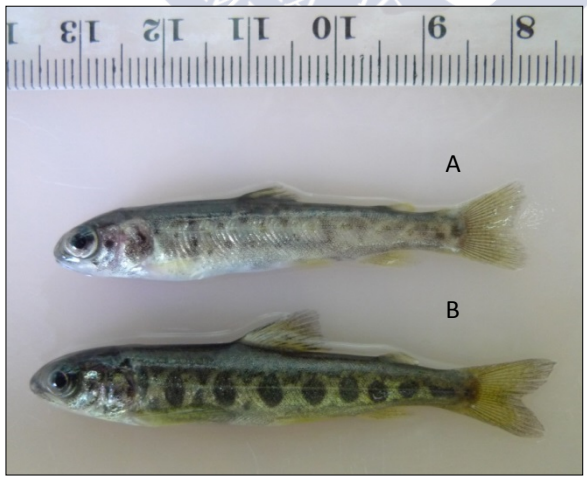

Figure 1.8.- Host fish juveniles form Galician rivers (A) *Salmo trutta* (brown trout) from river Ulla and (B) *Salmo salar* (Atlantic salmon) from river Eo. Photo by S. Lois.

1.3. Study Area

This work was carried out in the northwest region of the Iberian Peninsula, within the administrative boundaries of the autonomous region of Galicia. Galicia is a region that comprises 29,574 km^2 of the European continent (Figure 1.9). It has 1500 km of coastline bathed by the Atlantic Ocean and the Cantabrian Sea, and it shares Portugal's northern border.

The climate of Galicia is classified as thermo-oceanic with high precipitation values, exceeding 1000 mm per year in some areas, with warmer and drier areas with Mediterranean influence in the south-eastern portion. Landscape is hilly without sharp peaks, with a predominance of metamorphic and granitic rocks fractured by tectonic processes that confer generally lower pH values to surface waters (Rodríguez, 2001). There are two main lithological groups: granitic and metasedimentary (Loureiro & Matía, 2001) (Figure 1.10). Most of these materials are acid in nature and present a high fracturing degree due to tectonic processes. All these aspects influence the configuration of a river network made up of short rivers with limited basin surfaces and sudden changes in slope, which form an enclosed narrow valley (Rodríguez, 2001) (Figure 1.10). Rainfall causes marked seasonal changes in river flows. In Figure 1.9, the map of the study area is presented with some of the river names and Figure 1.11 example pictures of some of them (for more pictures and maps see Annex I). In Galicia there are 33 main river basins divided in 77 sub-basins (Rodríguez, 2001). The basin of river Miño is the largest in Galicia with a drainage area of 18,080 km^2 but as a general rule, river basins have a small size (Figures 1.11 and 1.12).

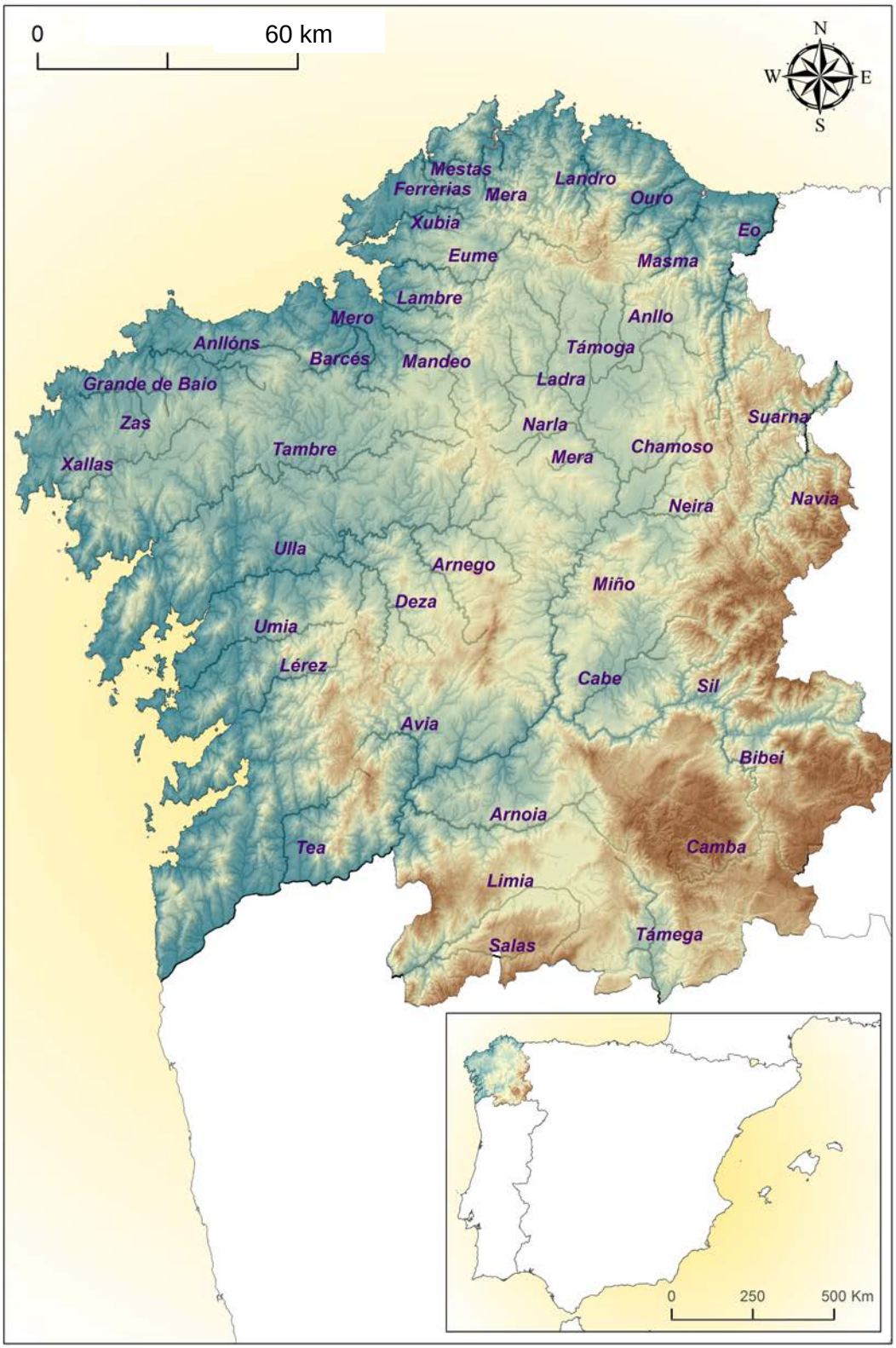

Figure 1.9.- Map of the study region showing river names.

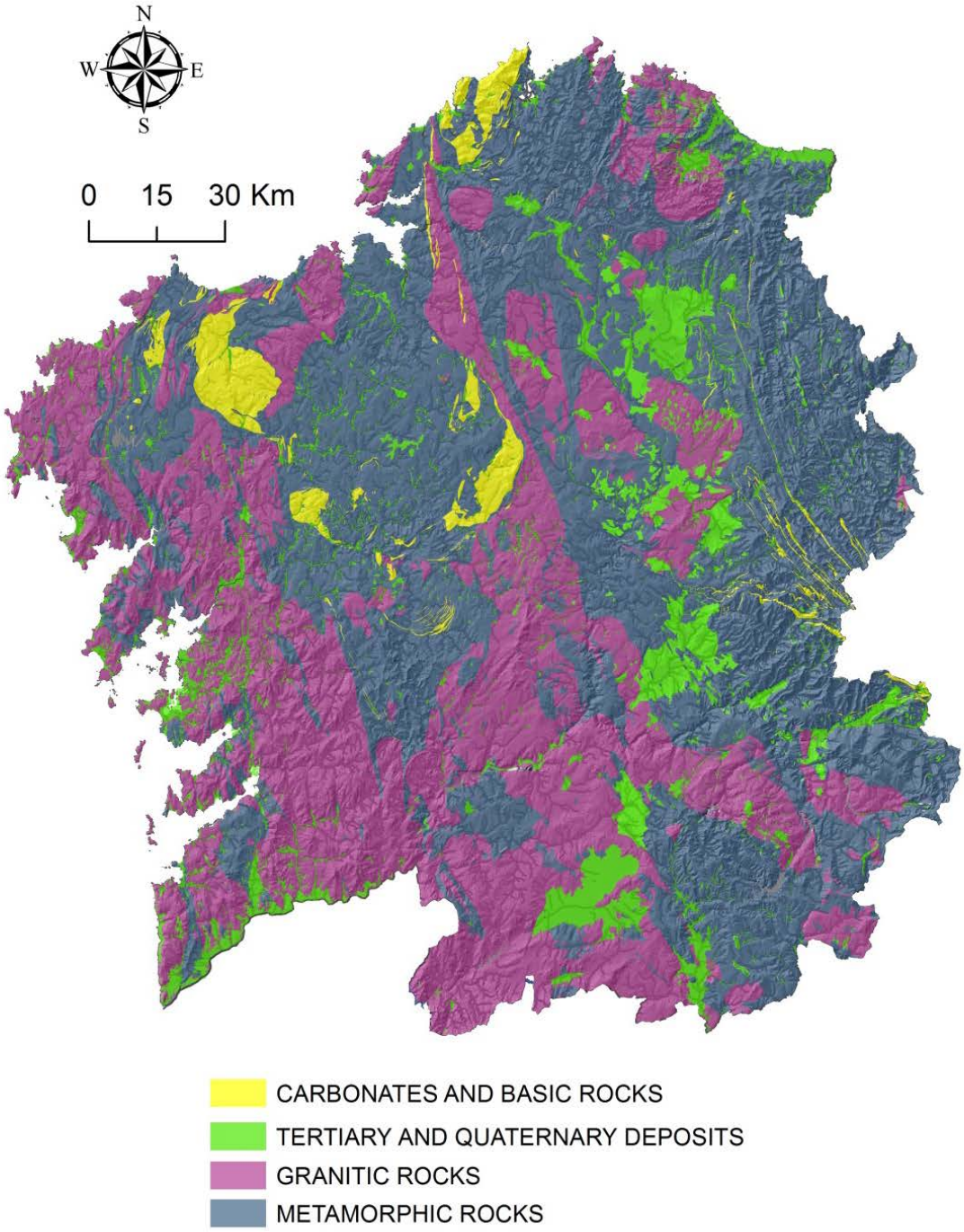

Figure 1.10.- Map of the main geologic features in Galicia. Modified from lithological map of Galicia, SITGA.

A

B

C

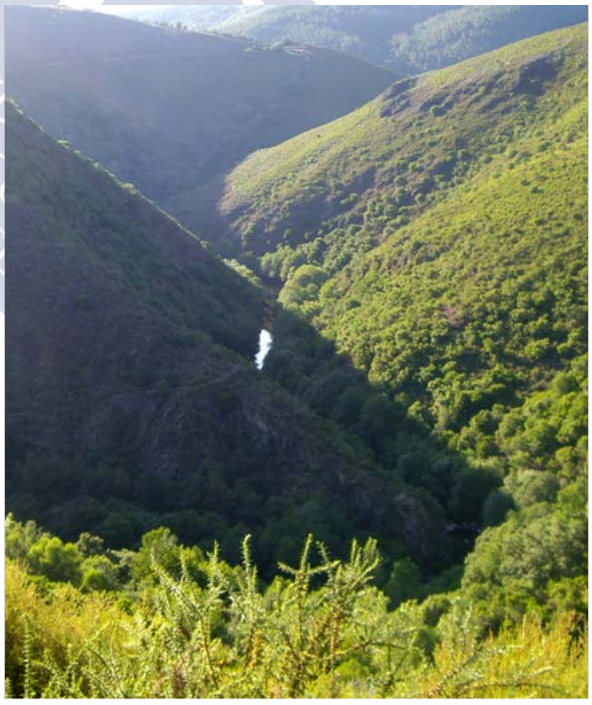

Figure 1.11.- Pictures of some rivers in the study area; (A) river Ulla, (B) river Navia and (C) river Navia valley. Photos by S. Lois.

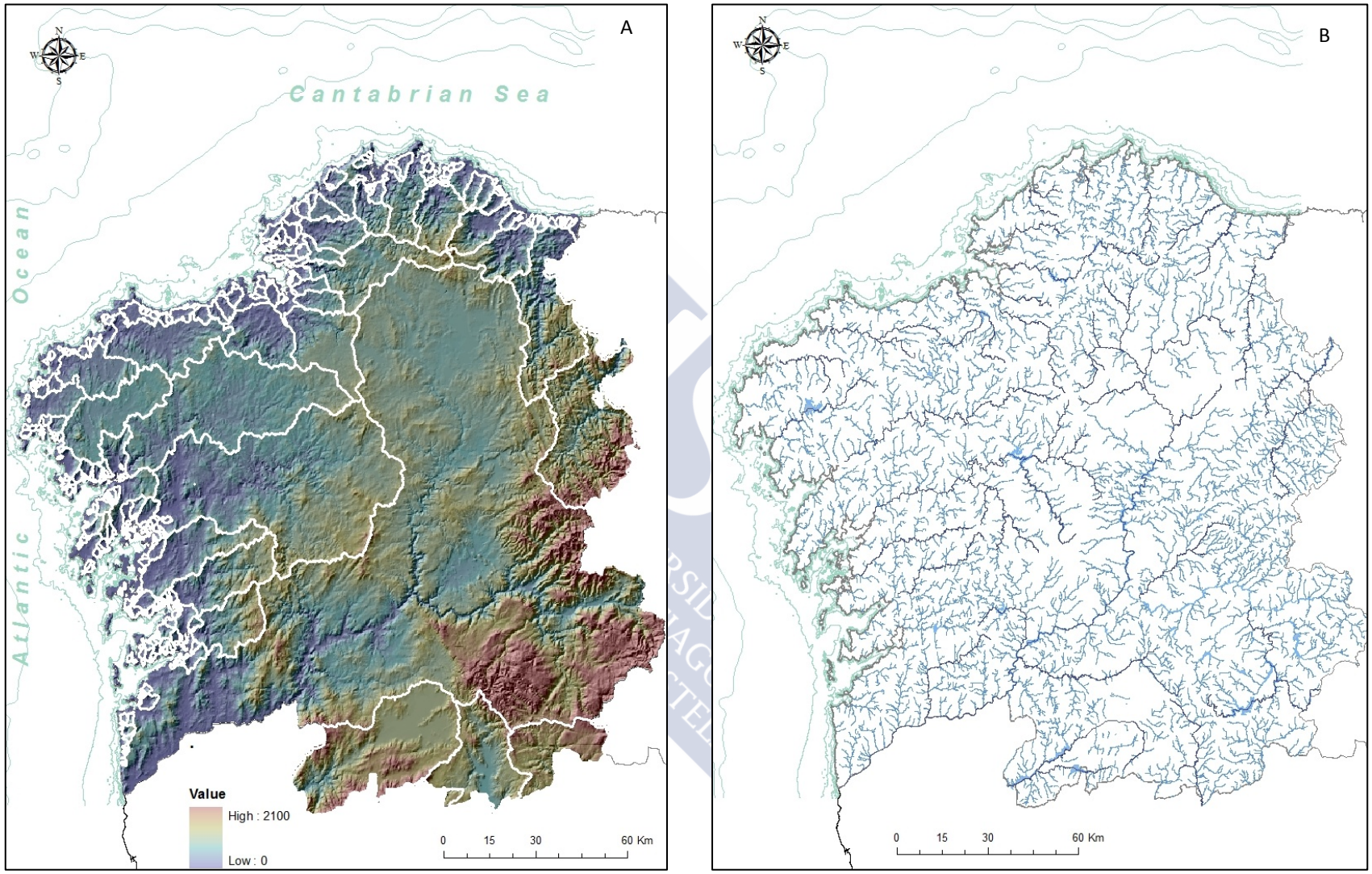

Figure 1.12.- Maps of the study region (A) drainage basin boundaries (white lines) and (B) river networks of the study area.

1.4. Objectives

The general objectives of this work are to provide a case study for sampling, modelling and prediction for freshwater species across and within riverine ecosystems. A broad-scale perspective with focus on biotic interactions is used to gain ecological and biological information of conservation value for *M. margaritifera*. In general, the techniques and processes described are applied to the endangered freshwater pearl mussel in different rivers in northwest of Spain, however the integrative process and the analytical techniques described in this study may be applied to any freshwater riverine species. In that sense research to address different hypotheses and objectives can be carried out by applying the processes described in this thesis for other species in different freshwater riverine environments of other geographical regions. Part of the content presented in this thesis is published in Lois (2010) and Lois *et al.* (2009, 2014, 2015).

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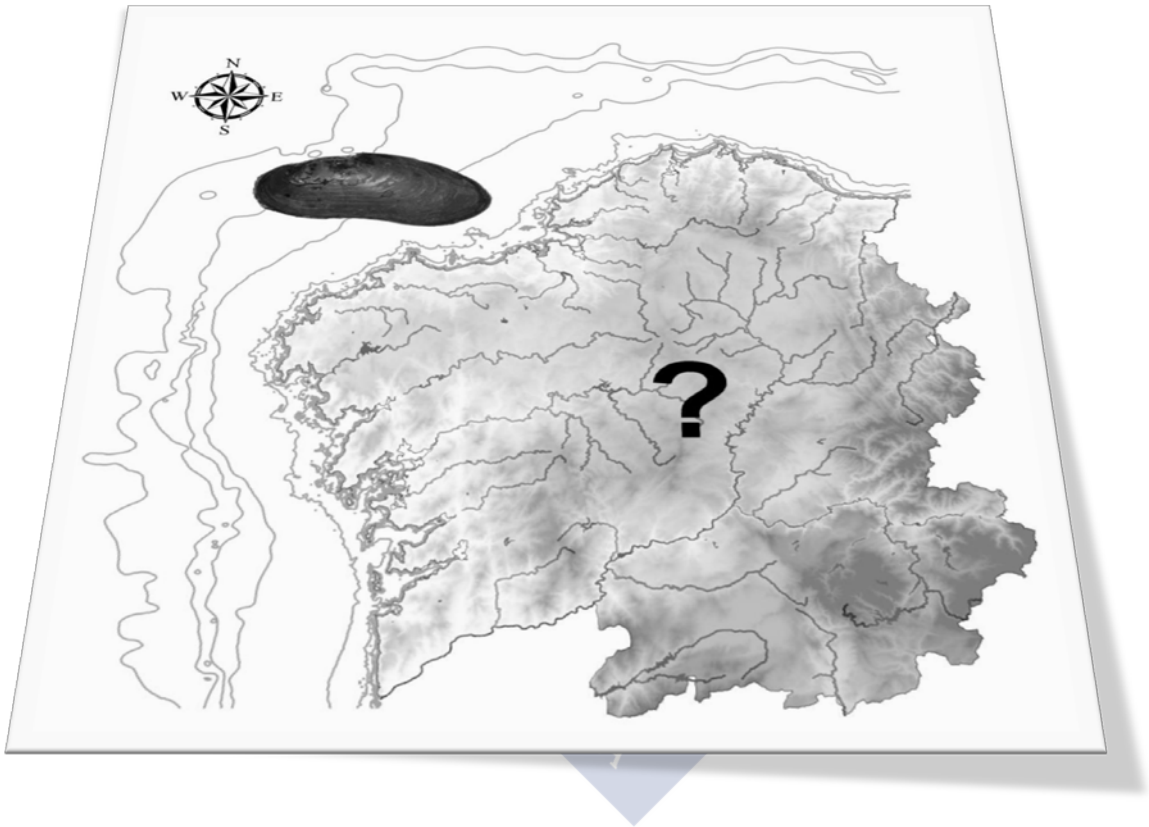
2. DISTRIBUTION AND ABUNDANCE ASSESSMENT



”Mentres auga leve o Miño, no Ribeiro non faltará viño”

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2. DISTRIBUTION AND ABUNDANCE ASSESSMENT

2.1. Introduction

As biodiversity loss continues unabated, increasingly more efforts and investments are made in conservation aimed at preventing species extinction. To maximize conservation success a sound framework including extensive baseline information is needed (Sutherland *et al*., 2004). Efforts to increase quantitative data about a species in its current habitats are crucial to obtain a complete picture of the species' ecology across its range (Fortin *et al.*, 2005). Learning more about a species distribution and its ecology will help to identify conservation units to attain successful integrative conservation efforts. Moreover, extensive field evaluations will help identify stressors and impairments to habitats and they are an important prerequisite to implement recovery strategies such as captive breeding (Snyder *et al.*, 1996).

M. margaritifera is considered a key species for the conservation of aquatic ecosystems (Geist, 2010) and is threatened with extinction throughout its geographic range. The species has been called one of the most threatened freshwater bivalves in the world (Machordom *et al.,* 2003). Moreover, in Europe populations of the freshwater pearl mussel have declined 90% during the last century (Araujo & Ramos, 2001), leading to the development of captive breeding techniques for freshwater mussels in Europe that are focused on this species (Gum *et al.*, 2011) in order to improve conservation efforts. Despite this early and extensive investment in captive breeding, knowledge about the species' ecological requirements is still fragmented and limited (Geist, 2010).

Although a few new populations of *M. margaritifera* have been recently found (Ostrovsky & Popov, 2011; Varandas *et al.,* 2013; Cosgrove *et al*., 2014), the most up-to-date synthesis of its distribution and status in Europe is that of Geist (2010). The northwest quadrant of the Iberian Peninsula appears to be the southern limit of the European distribution of the species. In Galicia, *M. margaritifera* was historically a common species (Macho, 1878). However, these historical data about the distribution

of *M. margaritifera are* limited (Macho, 1878; Azpeitia, 1933). Information about its distribution is fragmented and recent studies have focused on only a few populations and none of them were aimed at mapping its distribution in this area (Álvarez-Claudio *et al.*, 2000; Ziuganov *et al*., 2000; Araujo & Ramos, 2001; Grande *et al.*, 2001; Machordom *et al.*, 2003; San Miguel *et al.*, 2004; Bouza *et al*., 2007; Outeiro *et al.*, 2008). Thus, it is important to conduct extensive surveys to better define the contemporary distribution of the species in the southern area of its range. No previous study has aimed at knowing the complete distribution of this species in the extensive network of rivers in Galicia.

The objectives of the present chapter are to identify the current distribution of the species, estimate population densities and report on occurrence of small-sized individuals, which may be indicative of recruitment. Presentation of these data is intended to provide the necessary baseline for assessing the conservation status of *M. margaritifera* in the Galicia and to enable development of effective conservation efforts such as captive breeding, reintroductions and relocations.

2.2. Materials and Methods

The sampling methods commonly used for freshwater pearl mussel are based on both qualitative and quantitative methods (Bauer, 1986; Beasley & Roberts, 1996; Hastie *et al.*, 2000b; Álvarez-Claudio *et al.*, 2000; Cosgrove *et al.*, 2000; Young *et al.*, 2001b; Reis, 2003; Morales *et al.*, 2004; Rudzīte, 2005; Outeiro *et al.*, 2008; Österling *et al.*, 2010; Sousa *et al.,* 2014) using different designs depending on the objectives. The study area has many drainage basins and some contain an extensive river network. Rivers are typically short with small drainage areas. An extensive sampling plan is needed to search for presence of freshwater mussel species in as many rivers as possible in order to know the current species' distribution in Galicia. On the other hand, additional data about its abundance and recruitment are required to examine the condition of populations (Geist, 2010). Thus, a combination of qualitative and quantitative methods is the best option (Vaughn *et al.*, 1997).

The sampling methodology used was based on Villella & Smith (2005) for efficient estimation of freshwater mussel densities in large areas. It is a 2-Phase doubly-stratified sampling method that makes it possible to establish a relationship between quantitative and qualitative estimations. This strategy is also aimed at increasing the probability of locating areas with recruitment, as juveniles and adults can share the same habitat (Hastie *et al.*, 2000a; Hastie *et al.*, 2010).

Phase I: Qualitative Exploratory Sampling

Phase I field sampling served to search for *M. margaritifera* in rivers and estimate its abundance. For this phase, selection of rivers that could potentially contain the species were chosen with the following criteria: (1) the existence of bibliographic records of the species and/or (2) the presence of intermediate hosts of *M. margaritifera* (*Salmo salar* and/or *Salmo trutta*) and (3) maximizing the number of rivers sampled in Galicia. A total of 148 rivers and tributaries were selected. For field surveys each river was divided in sections 5 km long using ArcMap 9.3 (ESRI, 2009) (Figure 2.1). In each 5 km section two sampling points of variable length (between 50 and 200 m) were carried out using aquascopes to visually detect the species. In addition, the width of the river at each sampling point was measured with a laser rangefinder.

Previous work showed a pattern of river-bed distribution with half-buried but spatially segregated groups of mussels usually close to the banks (Outeiro *et al.*, 2008). Thus, for every sampling point in which *M. margaritifera* was found, individuals were counted, but only those present in a limited area of the river bed. This area was called “bank corridor”, defined as a corridor of the riverbed 2 m wide and 50-200 m long adjacent to the river banks where mussels were expected to be most abundant. From these counts, the density of individuals in each bank corridor was calculated and called “bank density”. Bank density of *M. margaritifera* was subsequently adjusted by procedures described in Phase II.

Once all selected rivers were explored, the sampling effort of Phase I was intensified in those rivers where the species was found. In each of these rivers, the 5 km section with the highest "bank density"was selected. This section was divided in 1 km sub-sections in which 2 or 5 sampling points were carried out (Figure 2.1). By sampling the areas with the highest abundances more thoroughly, the possibilities of finding individuals during these additional assessments increased, which was necessary to carry out Phase II properly (Smith, 2006).

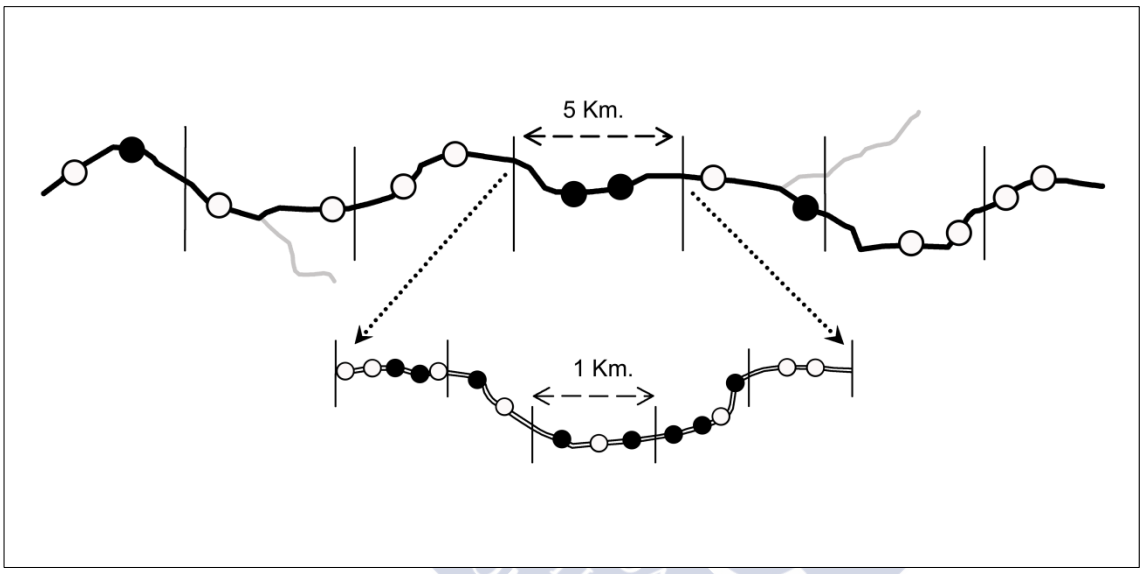


Figure 2.1.- In Phase I, rivers were divided into 5-km transects for surveys (upper). The area with highest density was further divided into 1 km sections and additional surveys were conducted. White dots represent sampling points where *M. margaritifera* was not detected and black dots represent presence.

Phase II: Quantitative Sampling

Phase II sampling focused on estimating the density of *M. margaritifera* in limited transects and on locating small-sized individuals that might be indicative of recruitment. Quantitative samplings were made at 25 sampling points which had already been examined in Phase I. Sampling point selection was done following two criteria: (1) we sampled points to represent areas of high and low density (Villella & Smith, 2005), and (2) sampling points were chosen to ensure representation across Galicia.

At each sampling point a fixed length river transect (50 m) was established; the sampling unit was a surface of 0.25 m^2 delimited by a metal frame. The number of sample units per transect ranged from 20 to 60 depending on the width of the river. The method used for sample collection was of two types, depending on the transect characteristics. In 3 cases, they were narrow river channels (mill channels or natural channels in braided river transects) where the sampling surface was relatively small and the mussels were spatially distributed as groups of individuals occurring along the riverbed with no clear aggregation pattern. In these cases, a simple random sampling was used. For the remaining 22 cases, sampling was carried out in river transects with a larger sampling area where the species was mostly distributed along one or both banks and less abundant in the middle of the river bed. In these cases stratified random sampling with optimal allocation was used (Krebs, 1999; Strayer & Smith, 2003). We defined two strata, one of them with high density and another with low density of mussels. Thus, by following the spatial distribution pattern of the species on the riverbed, a high density stratum was adjacent to the river banks. A scuba diving time based count of mussels at each stratum was made to calculate the number of samples by using optimal allocation. Once the number of 0.25 m^2 sample units of each stratum was determined, they were allocated in each stratum using coordinates. The coordinates of each sample were randomly chosen and laser rangefinders were used to locate their position in the field. All visible individuals within a randomly-located sample unit were counted. Subsequently, a hole 15 cm deep was excavated to detect the presence of juveniles, as they usually remain buried (Hastie *et al.,* 2000b). All individuals less than 65 mm long were counted and measured, as individuals of this size are generally considered to be juveniles (Bauer, 1986; Hastie *et al.*, 2000b; Young *et al.*, 2001b).

Using the counts of mussels in each quadrat sample we calculated the estimates of density and obtained average density by stratum and the weighted density for the transect following Krebs (1999) that uses the following calculation procedure:

$$X_{ST} = \frac{\sum_{h=1}^{L} N_h X_h}{N}$$

where X_{ST} is the total average density per sampling unit for the total of the transect, N_h the number of samples contained in stratum *h*, X_h is mean density for stratum *h*, and *N* the total number of sampling units. The variance of X_{ST} is calculated as

$$Var(X_{ST}) = \sum \left[\frac{W_h^2 S_h^2}{n_h} (1 - f_h) \right]$$

where S_h^2 is the variance of stratum *h*, f_h the sample fraction collected in stratum *h*, n_h the sample size collected in stratum *h* and W_h is the stratum weight calculated as the quotient between the stratum size and the total size of the sampling area.

The pattern of species distribution among samples was calculated for each stratum using Morisita's index of dispersion (I_d) (Morisita, 1959) by means of the following expression:

$$I_d = n \left[\frac{\sum X^2 - \sum X}{\left(\sum X\right)^2 \sum X} \right]$$

where n is the number of quadrats used in each stratum and X the sum of counts in each quadrat. The critical value *Mu* and the standardised Morisita's index (I_d) were obtained with the software EcoMeth (Krebs, 1999). Further details on the indices and on the estimation of population parameters is given by Krebs (1999) and Strayer & Smith (2003).

Finally, we found the relationship between the densities obtained in Phase I (bank density) and Phase II (transect density) for the 22 transects sampled in Phase II. This relationship between transect density and bank density provided a calibration adjustment of bank density to more accurately indicate density of mussels. Average density of mussels per river was used to estimate total population size in each river.

2.3. Results

Phase I

Of the 148 rivers and tributaries surveyed in the first phase of sampling, the species was not found in 91, its presence could be verified in 54 (Figures 2.2, 2.3 and 2.4) whereas only shells could be found in three (Table 2.2). A total of 2436 sampling points were examined distributed in 145 rivers (Table 2.1 and 2.2), which is a total of 350 km of examined river length. Of these sampling points, 555 (23%) have occurrences of *M. margaritifera*. Thirty-three previous records of *M. margaritifera* distributed in 26 rivers were confirmed with the present work (Table 2.2 and Figure 2.4). Moreover, the species was found in 522 new sampling points, representing a total of 54 rivers belonging to 23 river basins (Table 2.2). During this phase other unionoid species were found (Lois *et al.*, 2009) and the presence of the alien species *Corbicula fluminea* (Muller, 1774) was detected (Lois, 2010) (see Annex II for *C. fluminea* distribution map).

The density of individuals observed in the bank corridor (bank density) was calculated in the 555 sampling points in which the species was present. The bank densities values ranged from 0.003 ind m^{-2} to 31.25 ind m^{-2}.

	River	*Result*		*River*	*Result*		*River*	*Result*
1	Almofrei	–						
2	Anllóns	–	32	Grou	–	62	Pereiro	–
3	Arenteiro	–	33	Hospital	–	63	Queixa	–
4	Asma	–	34	Iso	–	64	Quintáns	–
5	Azúmara	–	35	Ladroil	–	65	Rao	–
6	Barbantiño	–	36	Lago	–	66	Ribeira pequena	–
7	Barbaña	–	37	Lama	–	67	Ribeiral	–
8	Barcala	–	38	Lañas	–	68	Rosende	–
9	Bateo	–	39	Lea	–	69	Santa Lucía	–
10	Belelle	–	40	Lengüelle	–	70	Sardiñeira	–
11	Beluso	–	41	Liñares	–	71	Sarria	–
12	Bidueiro	–	42	Lonia	–	72	Seco	–
13	Boente	–	43	Loio	–	73	Sil	–
14	Brandelos	–	44	Loira	–	74	Sionlla	–
15	Brandeso	–	45	Lor	–	75	Sor	–
16	Búbal	–	46	Louro (Miño)	–	76	Támega	–
17	Cabras	–	47	Louro (Ulla)	–	77	Tamuxe	–
18	Caldo	–	48	Maceda	–	78	Té	–
19	Caraño	–	49	Mao (Cabe)	–	79	Termes	–
20	Casoio	–	50	Mao (Sil)	–	80	Tioira	–
21	Castro (Baio)	–	51	Mariñán	–	81	Trabada	–
22	Cereixo	–	52	Mendo	–	82	Turia	–
23	Condomiñas	–	53	Mente	–	83	Uma	–
24	Conso	–	54	Miñor	–	84	Valga	–
25	Covo	–	55	Moia	–	85	Verdugo	–
26	Deva (Miño, Ourense)	–	56	Navea	–	86	Vilacoba	–
27	Deva (Miño, Pontevedra)	–	57	O Rosal	–	87	Vilaza	–
28	Dubra	–	58	Oitavén	–	88	Viñao	–
29	Edo	–	59	Oribio	–	89	Xares	–
30	Entíns	–	60	Ourille	–	90	Xunco	–
31	Ferreira	–	61	Pequeno	–	91	Zarzo	–

Table 2.1.- List of rivers where the presence of *M. margaritifera* was not detected in the first phase of sampling.

	River	*Result*		*River*	*Result*		*River*	*Result*
1	Arnego	+	20	Landro	+	39	Pambre	+
2	Anllo	+	21	Lavandeira	+	40	Requeixo	+
3	Arnoia	+	22	Limia	+	41	Rodil	+
4	Asneiro	+	23	Lodoso	+	42	Rosende	+
5	Avia	+	24	Lérez	+	43	Salas	+
6	Barcés	+	25	Maior	+	44	Tambre	+
7	Bibei	+	26	Mandeo	+	45	Tea	+
8	Cabe	+	27	Masma	+	46	Toxa	+
9	Camba	+	28	Mera	+	47	Trimaz	+
10	Castro (Xubia)	+	29	Mera (Miño)	+	48	Támoga	+
11	Chamoso	+	30	Mero	+	49	Tórdea	+
12	Deza	+	31	Mestas	+	50	Umia	+
13	Eo	+	32	Miño	+	51	Ulla	+
14	Eume	+	33	Narla	+	52	Xallas	+
15	Ferrerías	+	34	Navia	+	53	Xubia	+
16	Furelos	+	35	Neira	+	54	Zas	+
17	Ladra	+	36	Ouro	+	55	Roxán	shells
18	Lamas	+	37	Parga	+	56	Lengüelle	shells
19	Lambre	+	38	Reigadas	+	57	Castro de Lires	shells

Table 2.2.- List of rivers where presence of *M. margaritifera* was detected in the first phase of sampling.

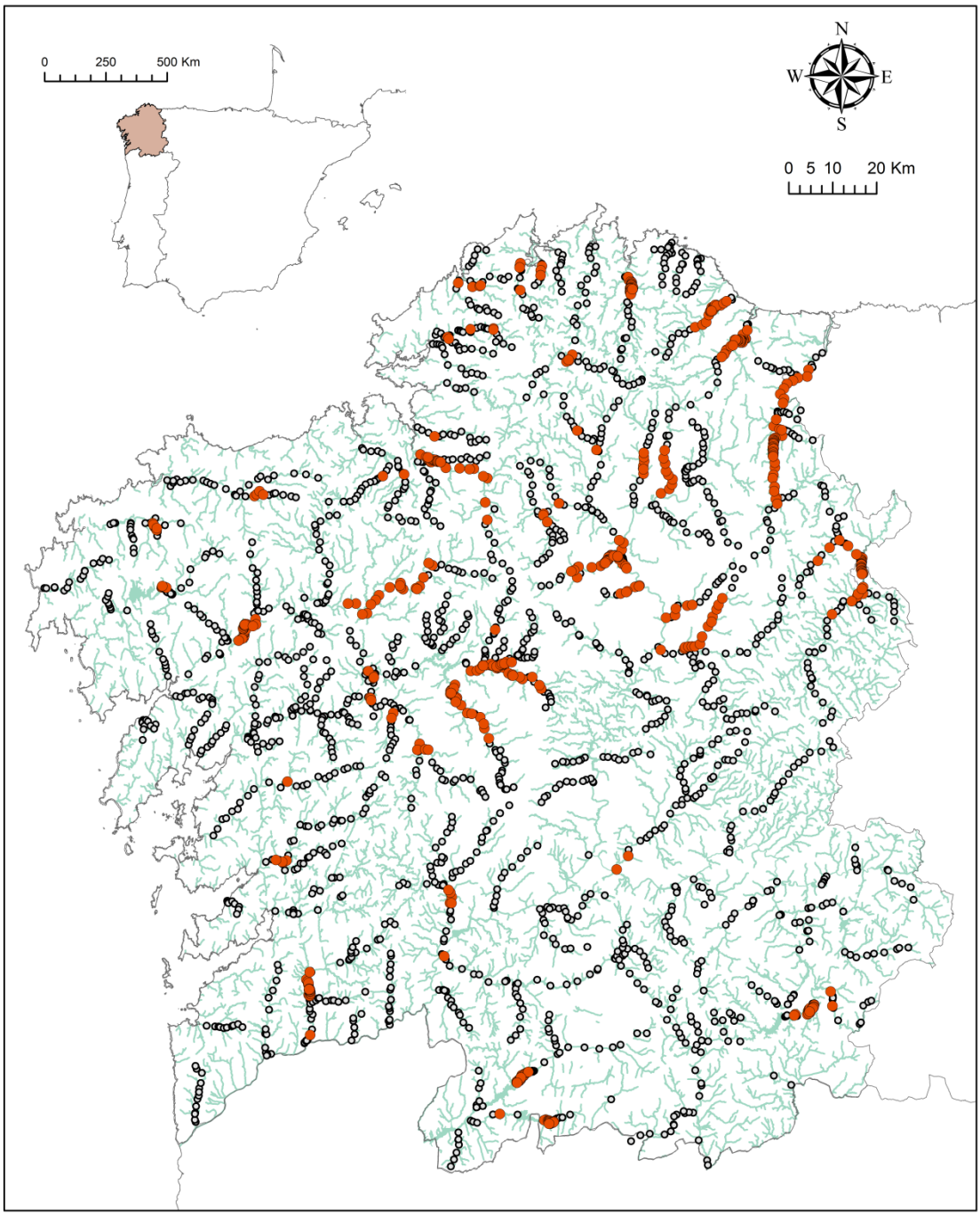


Figure 2.2.- Sampling results of Phase I. The grey dots represent sampling points where *M. margaritifera* was not detected (n=2436). The red dots (n=555) represent sampling points with presence of the species.

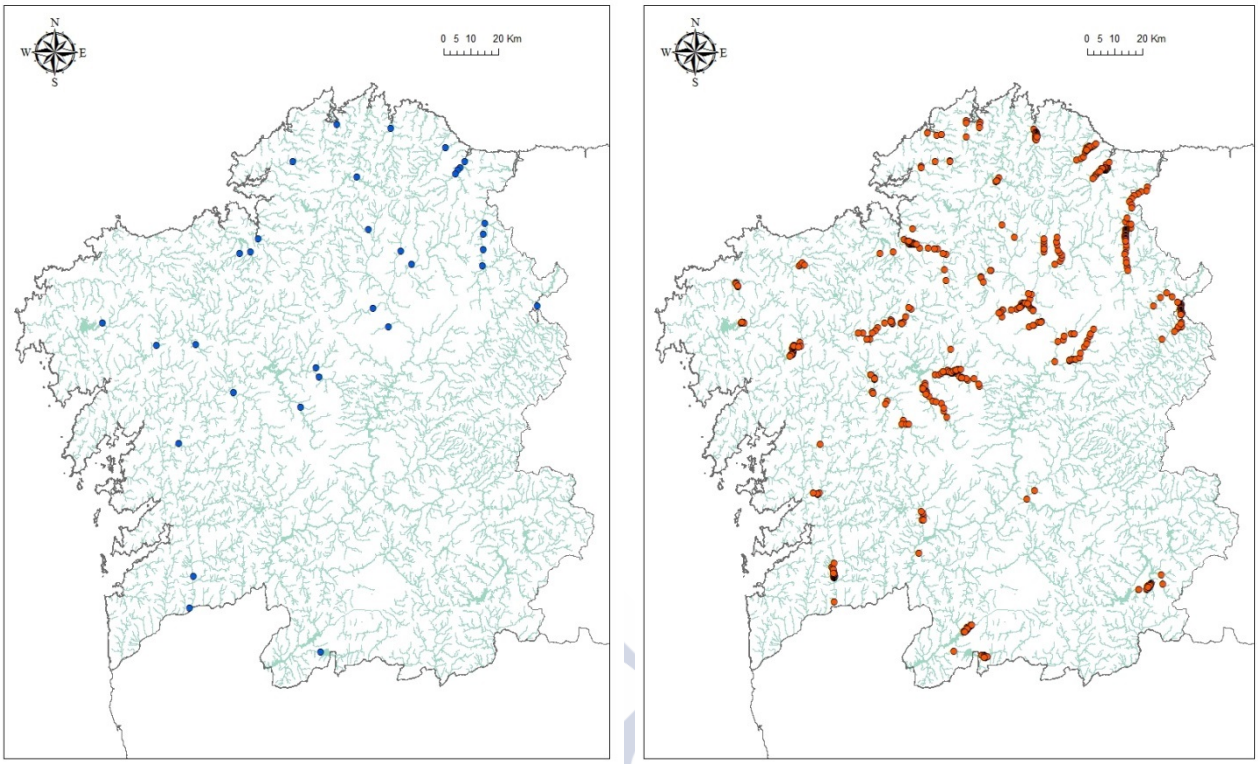


Figure 2.3.- Distribution map with presence records of *M. margaritifera*. Left map shows records documented in previous studies (blue dots). Right map shows current records (red dots).

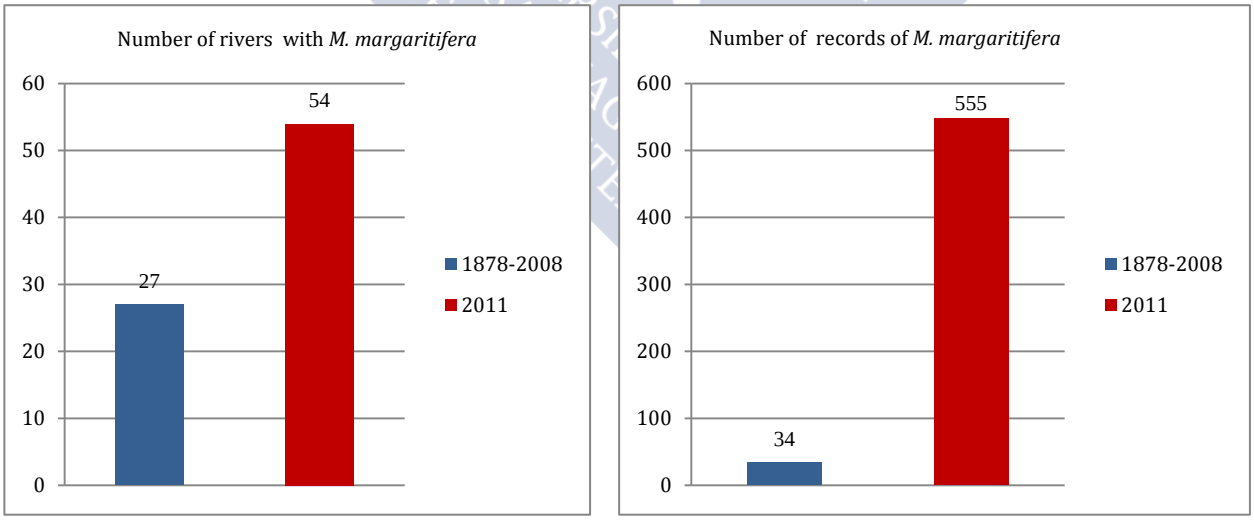


Figure 2.4.- Comparison between previous information and results from this study. Figure on the left: number of rivers. Figure on the right: numbers of records.

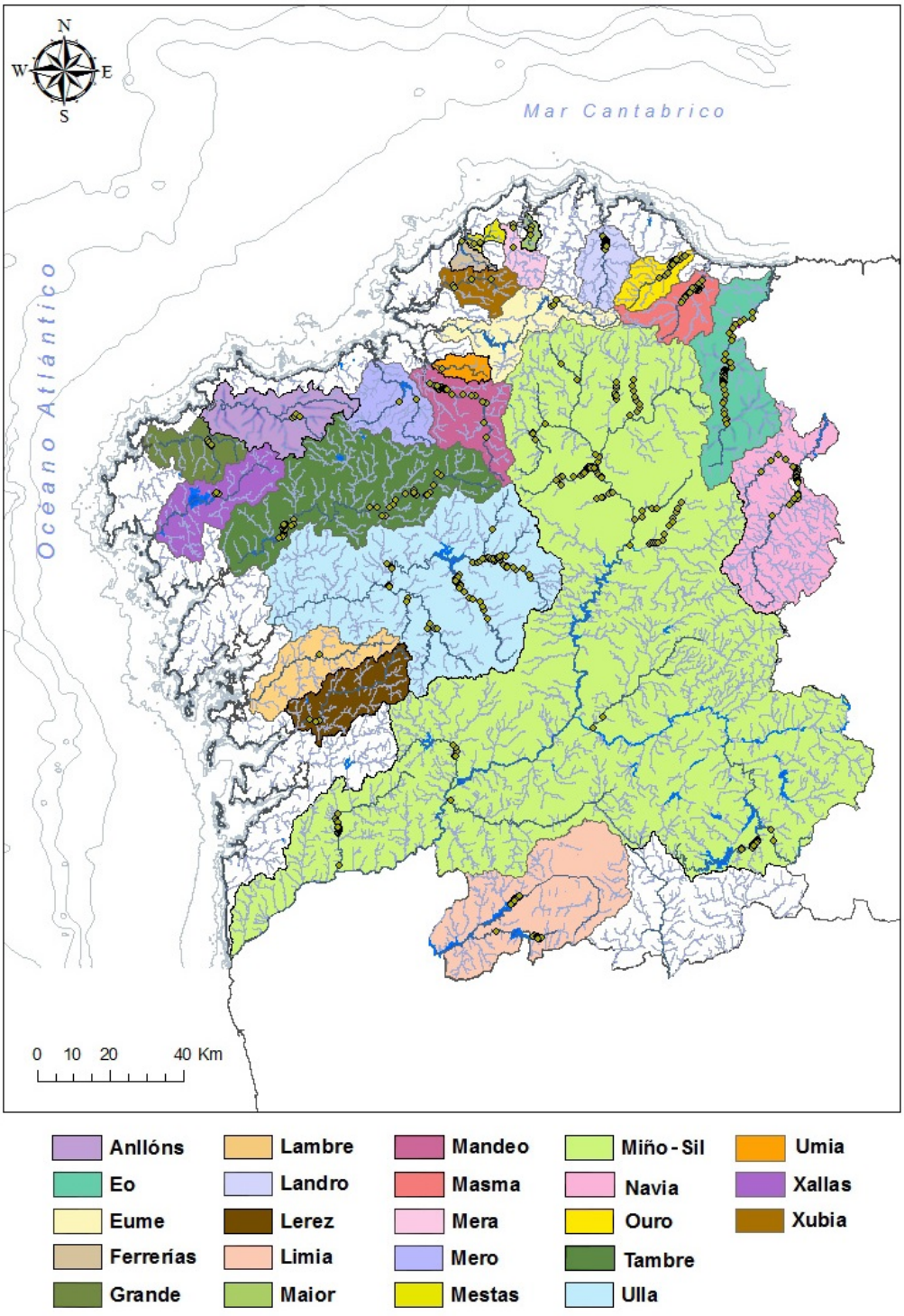


Figure 2.5.- Map of the drainage basins where *M. margaritifera* was recorded. Green dots show the presence of *M. margaritifera* and background colours display the basin drainage boundaries

Phase II

The density of *M. margaritifera* in the river transects (transect density as opposed to bank density in Phase I), obtained in the quantitative samplings of Phase II, ranged from 0.1 ind m^{-2} (River Ulla) to 21.02 ind m^{-2} (River Eo) (Table 2.3). However, the highest transect density was found in a channel of the Río Camba: 47.78 ind m^{-2}. On the other hand, the maximum density observed in a single sample also came from the channel of this river with 332 ind m^{-2}. Rivers Ulla and Arnego, where several quantitative samplings were carried out in different transects, showed highly variable density values, especially in River Arnego (0.3-8.9 ind m^{-2}). The transect with the highest number of individuals was in River Eo, where 20,135 individuals were found in an area of 943 m^2 (Table 2.3).

According to Morisita's standardised index (Ip), the individuals of *M. margaritifera* found in the high-density stratum and three channels, showed a spatial distribution pattern with mussels occurring in groups (*Ip*> 0, from 2.57 to 0.05) (Table 2.3). The spatial distribution was uniform in the high-density stratum (*Ip*= -0.04) in just one locality (Upper Ulla 3). In the low-density stratum, *M. margaritifera* also occurred in spatially segregated groups, although in some cases all the samples had no mussels so the density was zero (Table 2.3).

Sampling was also conducted to gain information about recruitment in Galician rivers. In only two rivers (Limia and Eo) individuals of *M. margaritifera* less than 65 mm long were found were found in high-density strata, in contrast at 11 sites they were found in the whole sampled transects (Table 2.4). Only 27% of these mussels <65 mm were buried, including two individuals less than 30 mm. No relationship was found between *M. margaritifera* density and presence of individuals <65 mm.

Transect Density

	HIGH DENSITY STRATUM					LOW DENSITY STRATUM				TRANSECT TOTAL			
River transects	***S (m^2)***	***D (ind m^{-2})***	***V***	***Ip***	***D max (ind m^{-2})***	***S (m^2)***	***D (ind m^{-2})***	***V***	***Ip***	***D (ind m^{-2})***	***σ***	***S (m^2) total***	***Total Nº***
Eo	100	115.5	2223.4	0.54	256	843	10.19	214.48	0.59	21.4	5.3	943	20135
Arnego 4	125	26.8	404.5	0.60	104	723	5.80	66.66	0.59	8.9	2.9	848	7541
Ouro	200	15.4	221.9	0.57	92	407	2.40	17.03	0.87	6.7	2.4	607	4049
Navia	100	51.7	944.7	0.53	236	1178	0.00	0.00	0.00	4.1	1.0	1278	5185
Salas	75	9.6	21.6	0.51	28	252	2.15	3.59	0.50	3.9	0.8	327	259
Mandeo	100	24.0	254.0	0.58	92	810	0.77	0.91	0.16	3.3	1.2	909	3027
Arnego 5	125	12.5	41.4	0.51	48	719	0.36	0.34	-0.07	2.2	0.5	844	1820
Arnego 1	200	6.9	27.3	0.55	40	580	0.31	0.30	-0.04	2.0	0.7	780	1550
Masma	100	10.8	36.2	0.52	44	861	0.61	1.03	0.52	1.7	0.6	961	1604
Narla	100	2.0	4.0	0.24	20	550	1.39	3.95	0.55	1.5	0.6	650	965
Limia	60	7.0	11.7	0.16	16	759	1.02	1.47	0.49	1.5	0.4	819	1195
Arnego 6	100	3.6	13.3	0.56	32	270	0.57	0.91	0.42	1.4	0.5	370	512
Upper_Ulla 5	100	5.9	28.8	0.53	44	445	0.24	0.24	-	1.3	0.4	545	699
Tambre	300	3.3	5.8	0.47	20	1905	0.57	0.70	0.23	0.9	0.3	2205	2089
Arnego 2	100	4.0	7.3	0.50	20	596	0.16	0.16	0.00	0.7	0.2	696	495
Upper_Ulla 6	150	1.1	1.2	0.08	12	625	0.43	1.29	1.00	0.6	0.4	773	431
Upper_Ulla 2	200	2.4	4.9	0.51	12	890	0.00	0.00	-	0.4	0.1	1090	480
Upper_Ulla 4	125	1.9	3.2	0.50	12	519	0.00	0.00	-	0.4	0.1	644	239

Arnego 3	100	1.0	3.0	1.00	12	341	0.14	0.14	0.00	0.3	0.2	441	149
Tea	200	2.6	5.5	2.77	24	1414	0.00	0.00	0.00	0.3	0.1	1614	516
Upper_Ulla 1	150	1.7	1.8	0.05	8	842	0.00	0.00	-	0.3	0.1	992	257
Upper_Ulla 3	125	0.3	0.3	-0.04	4	379	0.00	0.00	-	0.1	0.1	504	38

Channel density

Channels	***S (m^2)***	***D (ind m^{-2})***	***V***	***Ip***	***D max (ind m^{-2})***	***Total Nº***
Camba_C	287.0	47.8	15.43	0.52	332	13719
Landro_C	143.2	3.8	1.10	0.19	16	554
Tea_C	187.8	2.5	1.19	0.52	24	469

Table 2.3.- Results of sampling carried out in Phase II; (S) surface area , (D) mussel density, (V) variance, (Ip) standardised Morisita index and (σ) standard deviation.

	Length >50-65 mm	Length 30-50 mm	Length <30 mm	Total <65mm	% Samples
Navia	24	2	0	26	24
Eo	11	3	0	14	10
Tea_C	8	1	0	9	17.5
Limia	3	2	2	7	10
Salas	2	2	0	4	10
Upper_Ulla 1	4	0	0	4	8
Masma	1	2	0	3	6
Mandeo	1	1	0	2	5
Ouro	0	2	0	2	2.5
Arnego 4	2	0	0	2	2
Tambre	1	0	0	1	1.7

Table 2.4.- Number of individuals less than 65 mm long found in the quantitative samplings of Phase II.

Combining Phase I and Phase II Results

When comparing bank densities of Phase I with transect densities of Phase II for the same localities, a linear relationship was observed (Figure 2.6). This could be clearly seen in a double log transformation calibration curve that made it possible to adjust bank density estimates to those obtained in the more intensive survey estimates of density from Phase II. The regression of transect density on bank density (R^2= 0.81, P < 0.001) for 22 transects in 12 rivers allowed transect density to be estimated for all 555 sampling points in Phase I.

The density estimates for all sampling points was assigned a categorical value to represent one of four categories of density (Figure 2.7). In more than 50% of the sampling points (298) *M. margaritifera* occurred at low density (<0.1 ind m^{-2}), whereas densities were high or very high in 110 sampling points. Fifteen rivers had areas of high density and five rivers had areas of very high density. Mean density was calculated for each river (Table 2.5). Density estimates show high variability within and between rivers. The number of individuals present in each river (Figure 2.8) was also estimated

(Table 2.5). For the rivers in which presence of small individuals was observed, all were estimated to contain more than 3000 individuals. The basin with the most rivers containing *M. margaritifera* was the Miño despite having low numbers of individuals. The River Eo had the highest estimated number of individuals 43,334. It was estimated that there were 188,734 individuals of *M. margaritifera* in the 54 rivers of Galicia.

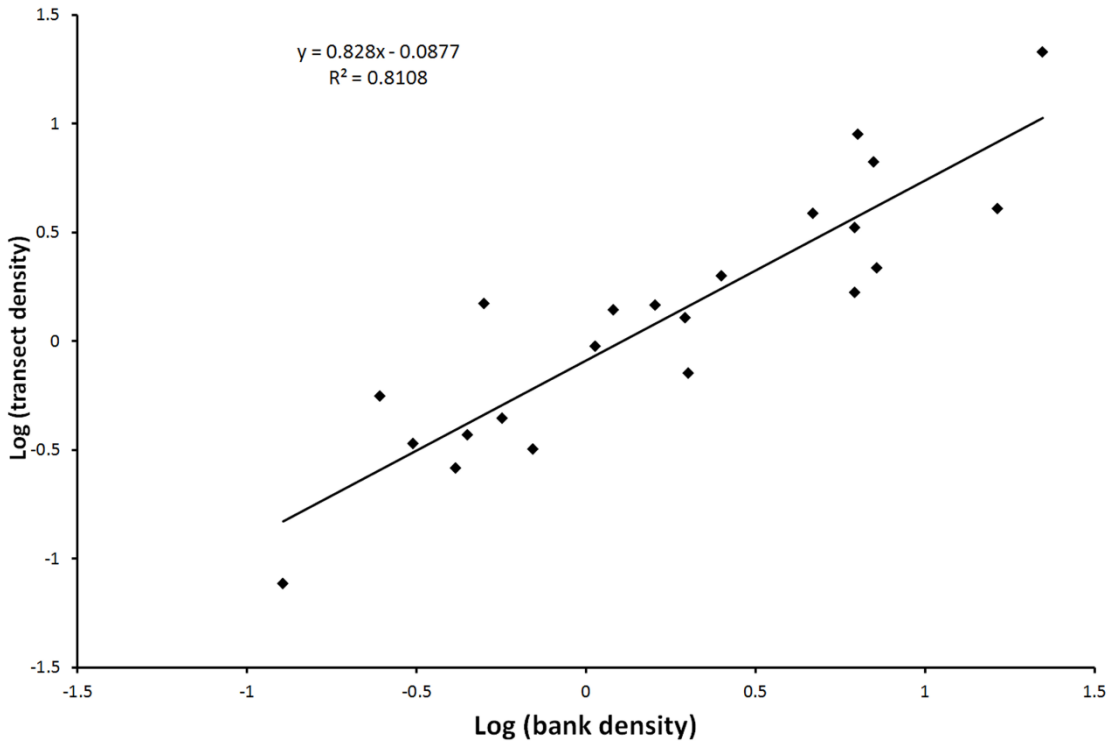


Figure 2.6.- Log-linear relationship between bank density of *M. margaritifera* (Phase I) and transect density (Phase II).

	River	Estimated individual No.	Surface (m^2)	Length (m^2)	N	Average	DS	Min	Max
1	Eo*	45334	42193	2565	*46*	1.341	3.550	0.007	21.400
2	Navia*	30555	33983	1667	*34*	1.290	2.740	0.072	14.120
3	Masma*	19405	20256	1740	*30*	0.889	0.860	0.021	3.700
4	Camba	15588	5695	651	*24*	2.430	9.510	0.019	47.780
5	Arnego	14085	13654	1240	*31*	0.620	1.626	0.014	8.900
6	Salas*	8482	28127	1130	*19*	0.660	1.110	0.014	3.860
7	Ulla *	7982	37000	3700	*43*	0.270	0.348	0.008	1.450
8	Landro	7703	13168	1860	*26*	0.567	1.051	0.011	3.860
9	Mandeo*	7569	21672	1445	*33*	0.397	0.853	0.008	3.700
10	Tambre*	7397	41530	2503	*45*	0.146	0.282	0.007	1.260
11	Narla	7333	14638	1865	*34*	0.498	0.560	0.007	2.230
12	Ouro*	6731	11390	1440	*25*	0.603	1.495	0.009	6.700
13	Tea*	3468	18752	1030	*21*	0.365	0.421	0.018	2.500
14	Limia*	3182	8244	615	*21*	0.347	0.421	0.018	1.500
15	Avia	859	7150	350	*4*	0.180	0.249	0.020	0.540
16	Támoga	578	4425	430	*6*	0.087	0.050	0.024	0.128
17	Tórdea	377	6138	935	*12*	0.069	0.070	0.015	0.228
18	Anllo	194	4160	620	*9*	0.043	0.038	0.012	0.129
19	Mera (Miño)	176	1825	520	*5*	0.078	0.046	0.022	0.143
20	Eume	158	1700	150	*3*	0.105	0.089	0.038	0.207
21	Lavandeira	137	895	180	*2*	0.158	0.179	0.031	0.285
22	Miño	137	4750	260	*5*	0.027	0.013	0.018	0.040
23	Ladra	98	8000	320	*2*	0.012	0.001	0.011	0.013
24	Xallas	98	4500	300	*6*	0.028	0.019	0.014	0.060
25	Chamoso	94	2630	490	*5*	0.047	0.026	0.016	0.080
26	Parga	91	2415	230	*3*	0.054	0.023	0.020	0.068
27	Suarna (Lamas)	86	7125	750	*5*	0.015	0.025	0.008	0.020
28	Ulla (medio)	82	1735	250	*6*	0.043	0.021	0.022	0.068
29	Furelos	75	250	50	*1*	0.310			
30	Deza	75	7500	500	*2*	0.011	0.005	0.007	0.014
31	Zas	64	1200	300	*6*	0.053	0.052	0.018	0.150
32	Rosende	59	1680	420	*4*	0.019	0.010	0.008	0.029

	River	Estimated individual No.	Surface (m^2)	Length (m^2)	N	Average	DS	Min	Max
33	Rodil	54	3000	200	*1*	0.018			
34	Asneiro	49	620	310	*3*	0.022	0.020	0.008	0.044
35	Mera	48	1125	150	*3*	0.032	0.013	0.018	0.040
36	Xubia	40	2325	310	*3*	0.017	0.013	0.010	0.030
37	Lodoso	33	375	50	*1*	0.090			
38	Maior	32	1440	740	*3*	0.038	0.030	0.008	0.068
39	Neira	26	3000	120	*1*	0.009			
40	Castro (Xubia)	25	960	240	*2*	0.026	0.025	0.009	0.044
41	Lérez	20	1275	170	*2*	0.017	0.009	0.010	0.024
42	Cabe	17	1350	250	*2*	0.013	0.008	0.007	0.018
43	Arnoia	17	1250	110	*1*	0.014			
44	Toxa	16	1875	250	*2*	0.008	0.000	0.008	0.009
45	Umia	16	900	100	1	0.018			
46	Requeixo	15	1500	200	*1*	0.010			
47	Bibei	13	1120	280	*2*	0.011	0.002	0.009	0.012
48	Mero	10	600	100	*1*	0.018			
49	Mestas	9	625	250	*3*	0.015	0.005	0.010	0.018
50	Barcés	8	900	120	*1*	0.009			
51	Lambre	8	750	100	*1*	0.010			
52	Trimaz	8	750	100	*1*	0.010			
53	Reigadas	7	480	120	*1*	0.028			
54	Ferreirías	7	280	70	*1*	0.024			
55	Pambre	4	400	100	*1*	0.010			

Table 2.5.- Estimated number of individuals, sampled surface area, length, number of sampling points in which the species occurred (N), average density per river (ind m^{-2}), standard deviation (SD), minimum density (Min) and maximum density (Max). The (*) indicates rivers with presence of individuals less than 65 mm.

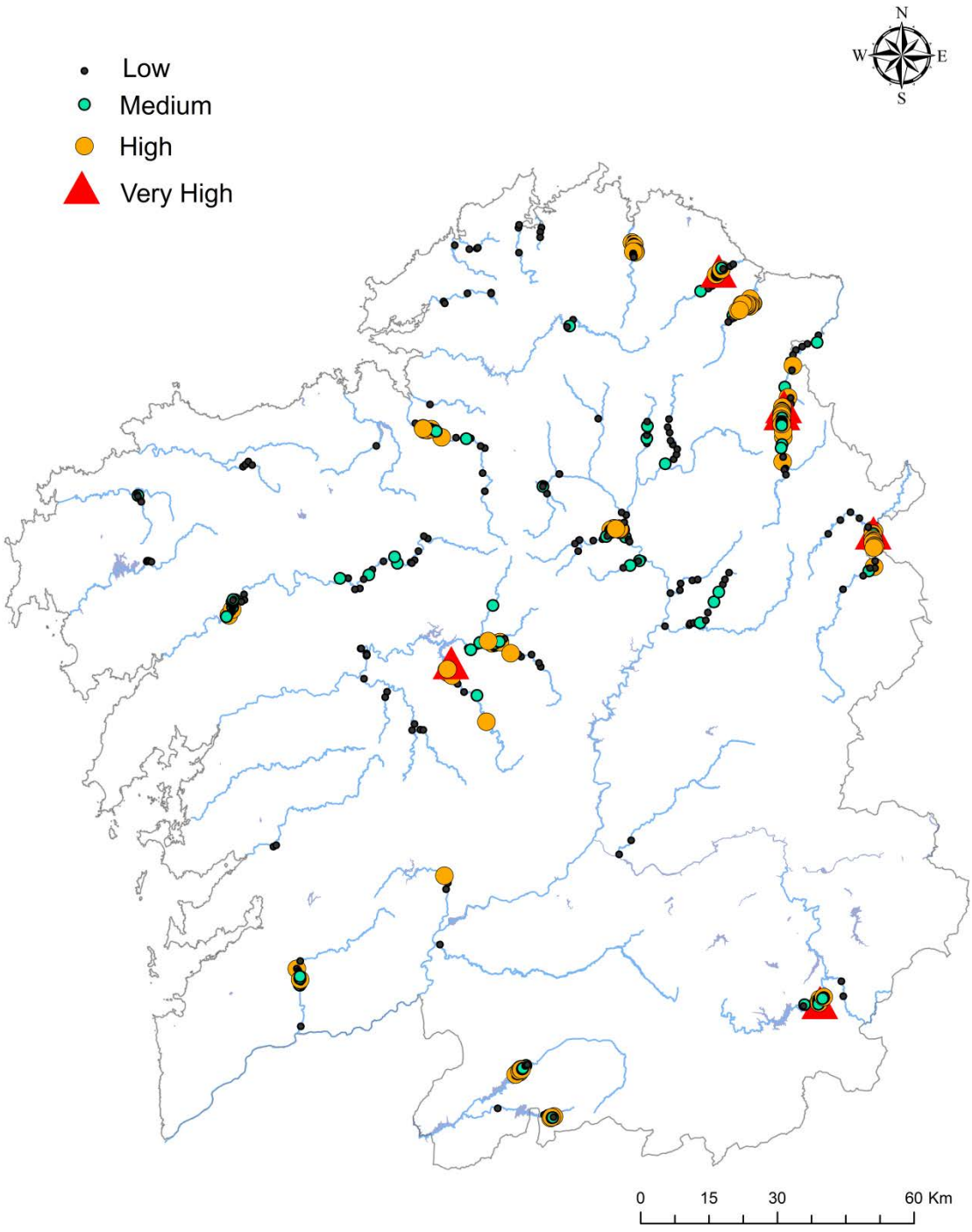


Figure 2.7.- Density of *M. margaritifera* in 54 Galician rivers. Symbols denote the four categories of density: very high (>5.0 ind m^{-2}), high (>0.5-5 ind m^{-2}), medium (0.1-0.5 ind m^{-2}) and low density (<0.1 ind m^{-2}).

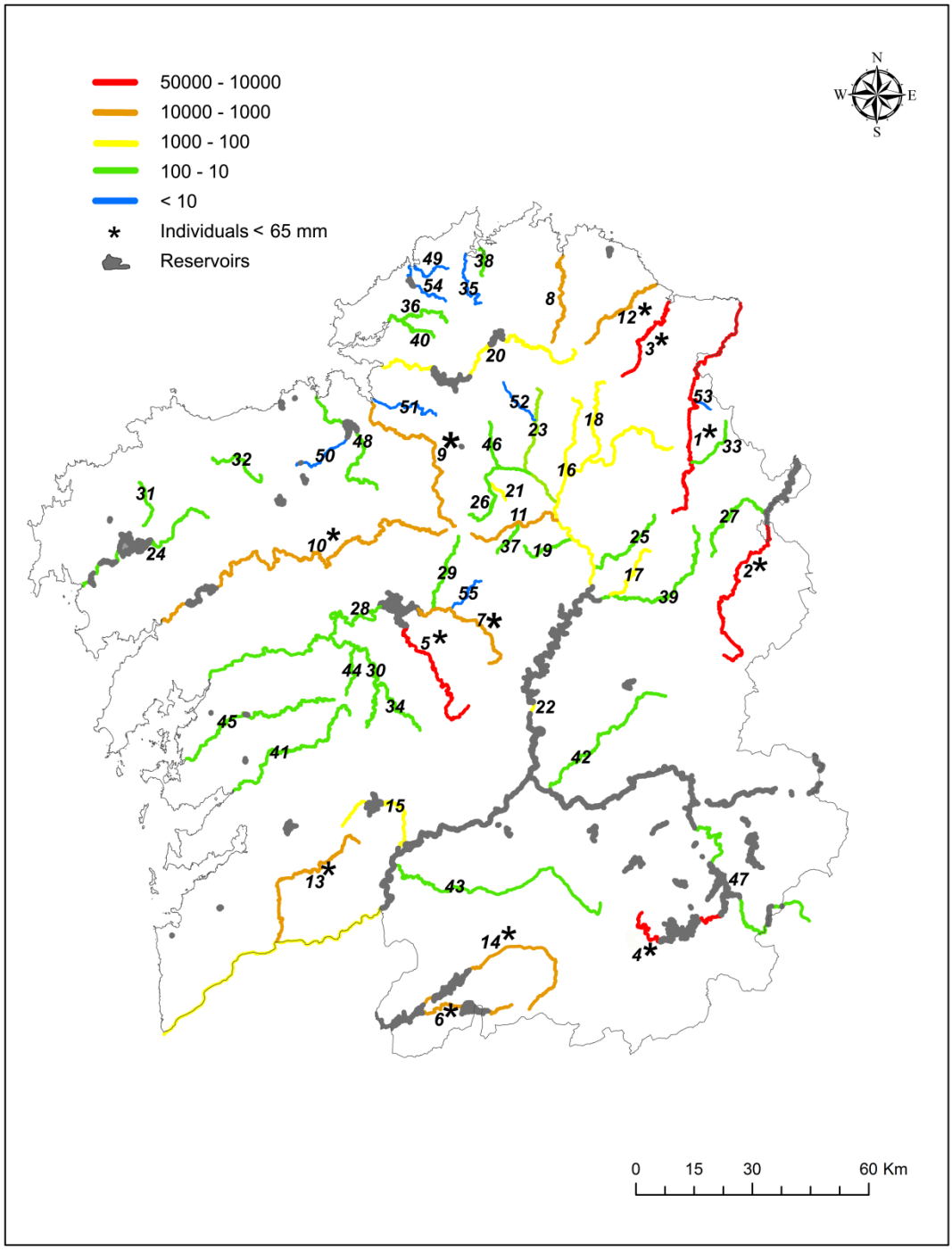


Figure 2.8.- Classification of Galician rivers according to the estimated number of individuals of *M. margaritifera*. Rivers where individuals <65 mm were observed are denoted with a (*). Numbers represent different rivers and tributaries (for more information see above Table 2.5).

2.4. Discussion

Most populations of *M. margaritifera* are known to be decreasing in number or have become extinct in Europe (Bauer, 1986, 1988; Ziuganov *et al.*, 1994; Beasley & Roberts, 1996; Beasley *et al.*, 1998; Cosgrove *et al.*, 2000; Araujo & Ramos, 2001; Young *et al.*, 2001a; Bespalaya *et al.*, 2007; Moorkens *et al.*, 2007; Geist, 2010). Populations of *M. margaritifera* have been found in 54 rivers of Galicia, 27 of which had not been documented before and information about 522 new localities with presence of the species has been obtained (Figures 2.2, 2.3, 2.4 and 2.5)

This work confirms species presence for all previous records for Galicia (Seoane, 1866; Graells, 1870; Macho, 1878; Azpeitia, 1933; Rolán & Otero-Schmitt, 1996; San Miguel, 1999; Ziuganov *et al.*, 2000; Araujo & Ramos, 2001; Grande *et al.,* 2001; Machordom *et al.,* 2003; Ondina *et al.*, 2003; Fernández de la Cigoña, 2004; San Miguel *et al.,* 2004; Bouza *et al.,* 2007; Araujo, 2008; Outeiro *et al.,* 2008). The presence of *M. margaritifera* in all large river basins of Galicia suggests that the species was broadly distributed in the rivers of this area in the past. This is supported by one of the few historical accounts in which the naiad is regarded as a common species in the rivers of northern Galicia, from Eo to Tambre (Macho, 1878).

The densities of *M. margaritifera* obtained in the 25 transects of Phase II ranged from 0.1 (River Ulla) to 48.7 ind m^{-2} (River Eo) (Table 2.3). Much of the data on densities of freshwater mussels are also based on the use of samples delimited by quadrats, but in most cases these samples are intentionally located in areas with the highest abundances, therefore their values are biased and only represent the density of specific areas of the river bed (Cawley, 1993). Thus, those data would only be comparable with densities we found in high-density strata (up to 256 ind m^{-2} in River Eo) or in mill channels (up to 332 ind m^{-2} in River Camba) (Table 2.3). These are high values, similar to those found in some of the greatest populations of the species, as those present in Russia (194 ind m^{-2} in Ziuganov *et al.,* 1994) or in Scotland (398 ind m^{-2} in Hastie *et al*., 2000b).

At present, the species is spatially distributed in a fragmented way, gathering only in specific areas of the river course. The estimated densities for the different sampling points of the same river show high variability as is the case of River Camba whose sampling points had values close to zero (0.019 ind m^{-2}) and up to 47.8 ind m^{-2} for instance (Table 2.3). In this context, Strayer (2008) pointed out that freshwater mussel populations and the places they live in are heterogeneous and fragmented. Although distribution and abundance patterns of populations are the result of the interaction among different causes (Hilborn & Stearns, 1982), in the case of unionoids, the connection among patches depends, above all, on the mobility and density of host fish (Arvidsson *et al*., 2012). This dispersion strategy makes it possible to link different patches, which enables, for example, the colonization of different areas and/or the recovery of patches with low densities (Strayer, 2008).

In Galicia, the connection among the different habitat patches along the river is severed by many dams, which limit the movement of the host fish and, in some cases, the access of salmon and sea trout to the mussel populations. Habitat fragmentation by dams is thought to be responsible for the extinction of salmon in some rivers of Galicia (Hervella & Caballero, 2002). It is notable that five of the Galician rivers containing small-sized individuals are still connected to the sea (Rivers Eo, Masma, Ouro, Mandeo and Tea, see Figure 2.8 and Table 2.4).

The genetic studies of *M. margaritifera* previously carried out in seven Galician rivers that are also included in the present paper, revealed a high degree of structuring and a reduced genetic intrapopulation diversity (Bouza *et. al*., 2007), which could be indicative of the low rates of gene flow. However, the discovery of additional populations and new sites of occupancy in Galicia will facilitate further broadening genetic and ecological studies of the species with the aim of getting deeper knowledge on the population dynamics of the species.

In general, the number of individuals present in a river and the recruitment rate are criteria used to assess the population status. According to some studies, a population of 5,000 individuals can be considered as “large” (Bauer, 1991; Hastie &

Cosgrove, 2002). In the present paper, 12 rivers have been found exceeding this number (Figure 2.8 and Table 2.5). Of all rivers examined, the River Eo has the largest population with more than 40,000 estimated individuals in the river. A total of 188,734 individuals of *M. margaritifera* are estimated to occur in the 54 rivers and tributaries of Galicia.

Individuals less than 65 mm long were detected in Phase I in three of the rivers sampled (Limia, Eo and Tea). However, quantitative samplings proved to be the most efficient for locating small-sized individuals, whose presence was confirmed in 11 rivers belonging to 10 different basins (Eo, Navia, Limia, Mandeo, Masma, Narla, Landro, Ouro, Tambre, Arnego and Ulla) (Table 2.4, and Figure 2.8). Individuals less than 30 mm were found in the River Limia (Table 2.4). However, the number of rivers with juveniles has probably been underestimated, as only quantitative samplings were carried out in a limited number of rivers. Hastie & Cosgrove (2002) and Hastie (2011) emphasized the difficulties detecting small-sized individuals on the riverbed, which leads to underestimation of the abundance of juveniles in populations. Individuals less than 10 mm long go unnoticed, even when using sieves (Young & Williams, 1984) and those less than 20 mm are difficult to find in many cases. Thus, Hastie (2011) suggests that a population should be considered to have recent recruitment when including individuals less than 30 mm. In Galicia, according to data obtained by Outeiro *et al. (*2008), only the River Eo was classified within this category. These results indicate that recruitment in the River Limia is also recent.

In general, individuals less than 65 mm were found in areas near the river bank. Despite being suggested that adults and juveniles may take up different habitats within the river bed (Geist & Auerswald, 2007), our results indicate that most individuals less than 65 mm shared the same habitat as adults, consistent with Hastie *et al.* (2000b). Despite its importance, there are presently no generalized criteria when assessing populations according to their type of recruitment. Thus, Cosgrove *et al.* (2000) regarded populations of Scotland as “functional” when they presented at least one specimen less than 65 mm, regardless of the population size, whereas Hastie

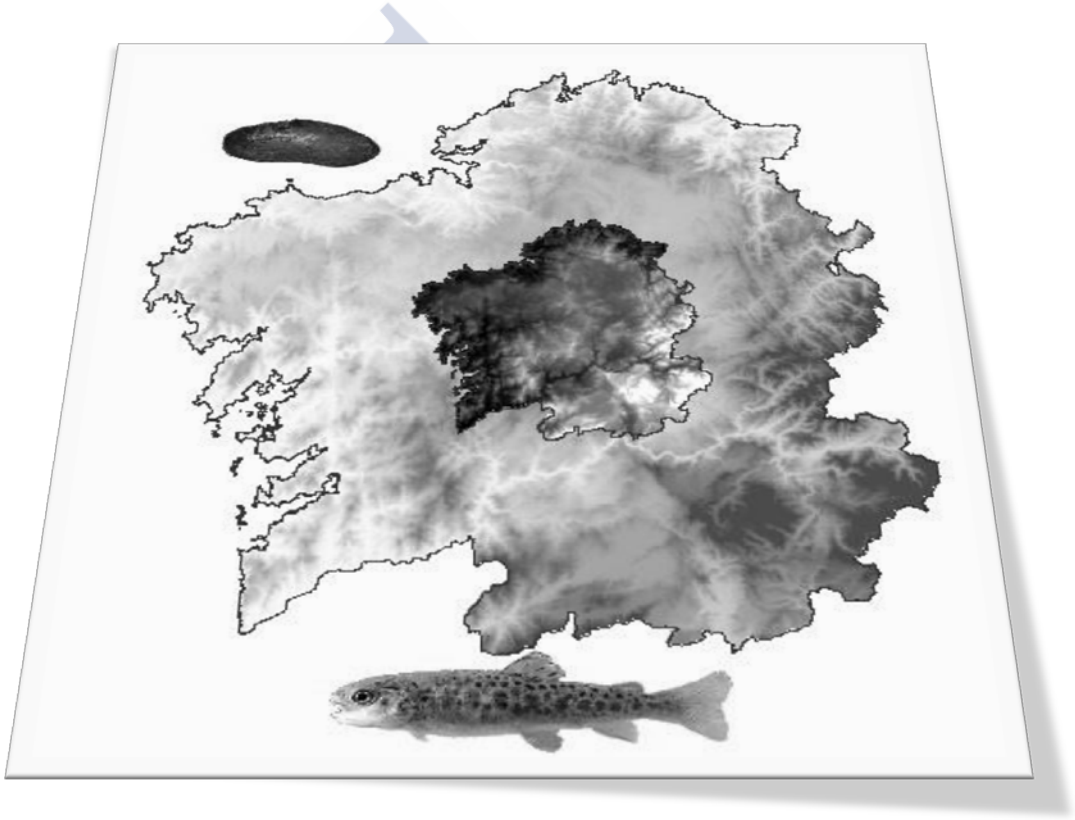
(2011) established a set of criteria for Scottish populations on the basis of presence percentages of individuals less than 65 mm with the aim of determining whether recruitment is sufficient to keep long-term populations. In order to assess recruitment in an objective way, it is necessary to take a definition of "juvenile" on the basis of the species biology (Cosgrove *et al.*, 2000). Galician populations of *M. margaritifera* have the highest known individual growth rates, the shortest longevity known and, according to the analysis of their growth pattern, they probably reach sexual maturity earlier than other populations (San Miguel *et al.*, 2004). Thus, in order to know if generation renewal is sufficient to keep populations, a demographic analysis is needed on the basis of the distribution of age frequencies (see Chapter 5).

The study area has the highest number of populations of *M. margaritifera* within the Iberian Peninsula and the total number of mussels estimated in its rivers exceeds that found in territories with greater surface areas including Germany (69 populations and 144,000 individuals), France (84 populations and a maximum of 100,000 individuals) and Austria (29 populations and 50,000 individuals) (Geist, 2010). Moreover, the apparent recruitment we observed in 11 Galician rivers is in contrast to the accounts that many European populations are not reproducing successfully (Young *et al.*, 2001a; Geist, 2010). Thus, the populations in Galicia are important for conservation of the species and they provide new opportunities to study its ecological requirements and genetic diversity especially in populations that are successfully producing small sized mussels.

Although peripheral populations usually take up less favourable habitats and show low and variable densities (Brown, 1984; Gaston, 1990; Brown *et al*, 1995), recent studies (Channell & Lomolino, 2000a,b) have pointed out that they may be very persistent populations over time. On the other hand, Hampe & Petit (2005) argue that populations at the periphery of a species range, especially those living in lower latitudes, such as *M. margaritifera* in Galicia, are of great relevance for the conservation of gene diversity and evolutionary potential of the species. This underscores the need for higher priority research and conservation of the populations.

The extensive baseline data reported in this chapter is used for the following chapters to study the ecological requirements and environmental limitations on *M. margaritifera* to provide a solid foundation for conservation planning.

3. MODELLING DISTRIBUTION BASED ON BIOCLIMATIC ENVELOPE AND BIOTIC INTERACTIONS



”Un non é ningún e dous son un”

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UNIVERSIDADE
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DE COMPOSTELA

3. MODELLING DISTRIBUTION BASED ON BIOCLIMATIC ENVELOPE AND BIOTIC INTERACTIONS

3.1 Introduction

A major challenge in ecology and resource management is to learn how to aggregate, retain and simplify the essential information necessary to understand complex patterns in ecosystems (Levin, 1992). This task can be especially challenging with distribution data of endangered species, because there may be many natural and human-related limiting factors that cause endangerment. Spatial habitat heterogeneity, discontinuities, variable human-impacts, and temporal variation are all potential sources of spatial complexity.

A variety of modelling approaches using machine learning methods are currently available and they are widely used to deal with spatial complexity in a species distribution (Warren & Seifert, 2011). There is sharply increasing growth in the application of MaxEnt (Phillips *et al.,* 2006; Phillips & Dudík, 2008), a machine learning method that utilizes the principle of entropy maximization. MaxEnt is often successful in predicting a species presence using model "features" developed on environmental measurements.

A multidimensional space of factors and their spatial and temporal interactions might need to be considered to understand species distribution to interpret this information into conservation value. Apart from the widely used abiotic factors, the so-called bioclimatic envelope (Araújo & Peterson, 2012), there is a need to include biotic interactions between species to obtain better species distribution models (SDMs) (Wisz *et al.,* 2013).

Biotic interactions that might influence species distribution include predation, competition, parasitism, and range limits of prey or mutualistic species (Wiens, 2011). Although biotic interactions are considered to be important at a local scale (Huston, 1999; Pearson & Dawson, 2003), they have received little attention at landscape, regional and continental scales (Wisz *et al.*, 2013). Interspecific interactions may affect

species' responses to abiotic factors across space and time, and they may have cascading effects on habitats, resource availability and accessibility and thereby jointly affect distribution patterns of two or more species (Boulangeat *et al.*, 2012).

An open challenge area in ecology is finding new ways of adding more ecological theory in SDMs (Austin, 2002; Elith & Leathwick, 2009) and presently the role of biotic factors in shaping biogeographic patterns is largely unexplored (Wiens, 2011). There is growing recognition that the relatively new endeavour of including biotic interactions in distribution models will benefit from additional examples (Araújo & Luoto, 2007; Heikkinen *et al.*, 2007; Schweiger *et al.*, 2008; Kissling *et al.*, 2012). Especially needed are analyses with broad scale approaches with fine-grained extensive field data of species occurrence (Wisz *et al.*, 2013).

The freshwater pearl mussel is a long-lived invertebrate with a specific life history that depends on a vertebrate host for survival. The study of its distribution illustrates a complex situation to be modelled: an endangered species that depends on the interactions with a host fish in a heterogeneous human-altered landscape, however it also provides an example of addressing complexity and biotic interactions when modelling distribution.

In this chapter the current distribution of *M. margaritifera* is analysed by using Maximum Entropy Modelling (MaxEnt for short) (Phillips *et al.*, 2006; Phillips & Dudík, 2008; Elith *et al.*, 2011) to predict probability of species occurrence. Four categories of broad scale environmental predictors are used (geologic, climatic, landform, and biotic factors) to develop model predictions of species distribution. In particular, this study is focused to address the question if biotic factors improve models of distribution of *M. margaritifera* to address its conservation.

3.2 Materials and Methods

Data Field Surveys

Field surveys were carried out in 148 Galician rivers and tributaries (2008-2011) to detect the presence of the species and to estimate its density (data described in Chapter 2) where a total number of 2436 samplings were made and *M. margaritifera* was observed in 555 samples, 54 rivers, and 23 river basins in Galicia. These results show that Galicia contains most of the populations of the species in southern Europe. To conduct the present work we began with 555 records of presence and eliminated records close to the border limits of Galicia and those with a geo-reference error of more than 200 meters, yielding initially 512 records of presence and species density for analysis belonging to 20 basins. The final set of records used in spatial analysis was reduced to 435 because of missing data for several predictors (Figure 3.1).

Environmental Predictor Variables and Scale

The study region (Figure 3.1) was divided into cells of 40x40 m. The pixel size was selected based on the minimum length of the survey sampling in which the species was found. All the layers were displayed in the same coordinate system using Universal Transversal Mercator projection and European Datum 1950 Zone 29N. Each layer was converted to the same spatial resolution and extent using ArcMap 9.3® and the Spatial Analyst Tools extension (ESRI, CA, USA). The digital model of the land at 40x40 m of resolution was used as the basis for layer calculations for every variable. Additional details are given below for each environmental predictor variable.

A broad scale approach was selected to design a set of environmental predictor variables that represent the whole river landscape of Galicia. Variable selection was based on existing knowledge about *M. margaritifera* ecology, relevance in characterizing the river systems of Galicia, and availability of landscape scale information for the region. Sixteen predictor variables were selected that belong to four categories: climate, geology, land form and host fish (Table 3.1).

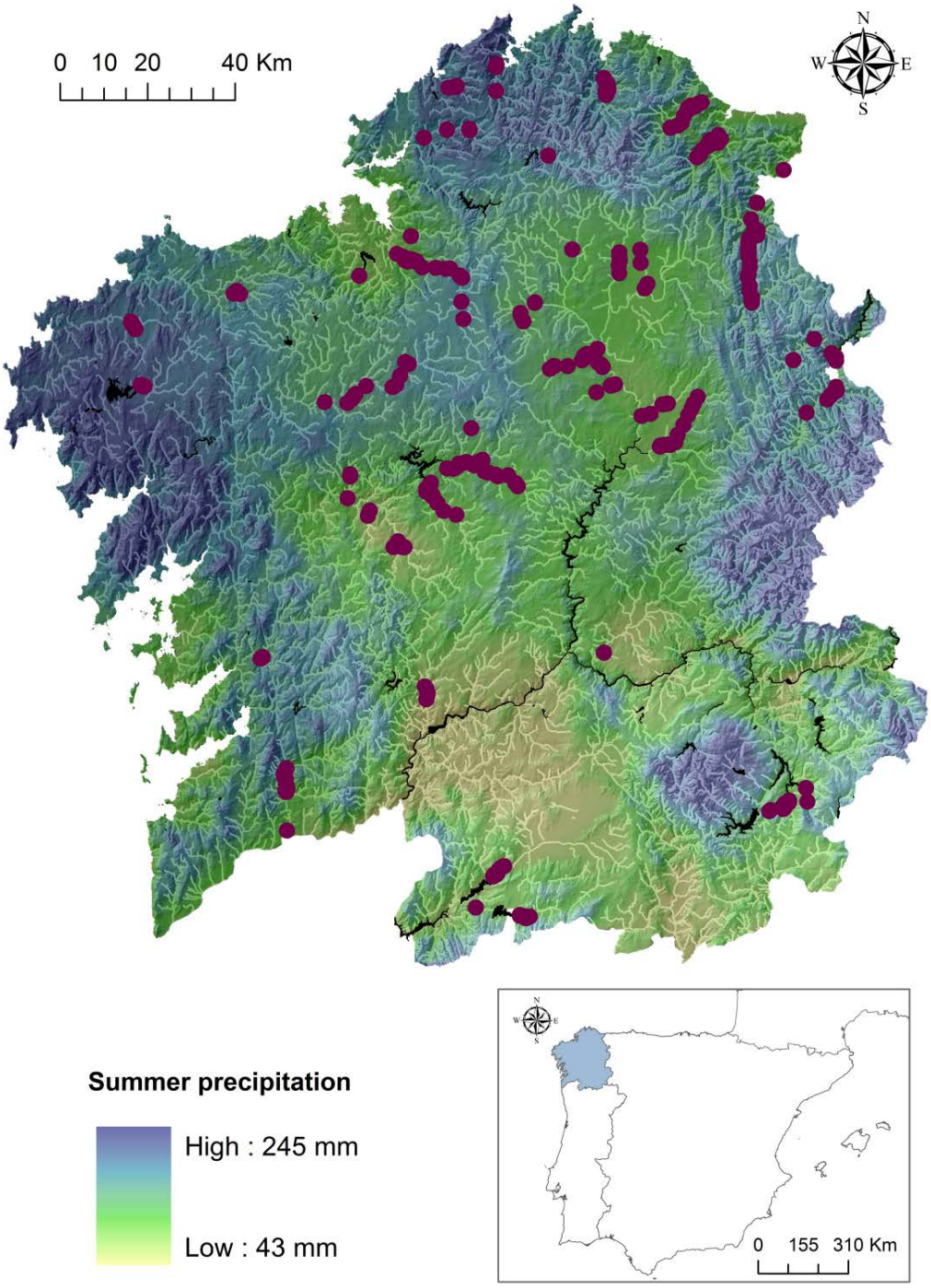


Figure 3.1.- The study region and the records selected for modelling, which includes 23 river basins in the northwest of the Iberian Peninsula. Locations of field surveys for *M. margaritifera* are shown by dots to indicate points where the species was present, black lines denote the largest reservoirs. Background colours represent levels of summer precipitation (July-September).

Climate data for Galicia were extracted from the Digital Climatic Atlas of the Iberian Peninsula. These data were obtained for a model of 200 m resolution with annual and monthly records from 2285 meteorological stations spanning 50 years (Ninyerola *et al.*, 2005). Six climate features that were considered relevant for *M. margaritifera* were extracted [average annual temperature, average summer (July-September) temperature, minimum and maximum summer temperature, average annual precipitation, and average summer precipitation]. Data layers were resampled using the nearest neighbour approach in ArcMap.9.3.

Categories in the surface geology map of Galicia (SITGA, 1:50.000) that may have relevance in characterizing the river systems and the ecology of freshwater pearl mussel were classified in 3 main geological categories: granitic, metamorphic, and detrital (tertiary/quaternary sedimentary deposits). These data were converted into raster layers of categorical values. Elevation and slope were extracted from the digital model of the land (MTD) at 40x40 m resolution from Spatial Data Infrastructure of Galicia (SITGA). Forest cover was reclassified and converted to a categorical feature in a raster file format from the data in CORINE Land Cover (CLC, 2000) vector data set by the European Environmental Agency.

Information on host fish for *M. margaritifera* (Atlantic salmon), resident trout and migratory trout) were obtained from the Fish Database of European Streams (Beier *et al.*, 2007) provided by the Galician government (Xunta de Galicia). These data included 1063 sampling points (1993-2003) across the majority of Galician streams. Four host fish predictor layers using the following information in this dataset: density of each of the three host fishes (ind m^{-2}) and biomass of salmonids (g m^{-2} per year) were designed. Vector values of host fish densities in each river were converted to a range of 40x40m raster pixel values using kernel density estimation implemented with ArcMap, using Hawth's Analysis Tools extension (Beyer, 2004) to assign density values to various pixels in a river.

Modelling Approach

A presence only modelling approach was used to gain understanding about the ecology of *M. margaritifera*. Presence only modelling was an attractive approach because contemporary presence records of a relatively immobile and long-lived species can be particularly informative about habitat requirements and distribution patterns, even when present conditions may limit population growth. The presence of the naiad can be an indication of suitable habitat conditions for the species over a long time period preceding the present. An approach also utilizing absence of the species was less attractive because there is insufficient historical information in Galicia to distinguish between real absences and local extinctions. Moreover, there may be suitable areas for the species in which it may be not present because a population can be extirpated by a transient event and the river subsequently can regain suitable conditions for the naiad. Consequently, a presence only approach was implemented in an effort to gain understanding of the ecology of *M. margaritifera* across a broad-scale region.

MaxEnt was the modelling approach selected because it is widely used for inferring species distributions and environmental tolerances using presence records (Warren & Seifert, 2011). It is a machine-learning method that uses an entropy maximization algorithm to characterize a species distribution in the environmental space by relating observed occurrence localities to a suite of environmental attributes where the species actually lives (Elith *et al.*, 2006b; Pearson *et al.*, 2007). This approach has been demonstrated to be a good method for predicting species presence with small data sets and with hidden complexity in empirical data (Elith *et al.*, 2006a; Graham *et al.*, 2008; Phillips & Dudík, 2008).

Modelling Processes of Species Distribution

MaxEnt version 3.3.3 (Phillips *et al.*, 2006) was used to predict *M. margaritifera* distribution across Galician rivers. MaxEnt auto-features were selected and the maximum of iterations was increased to 5000 to ensure convergence. Cross-validation

with 10 sample runs was applied. Logistic format was selected for the MaxEnt output to obtain values for probability of species presence that ranged from 0 to 1 in the gridded sample region. The analysis of species distribution was made in the whole landscape but for visual simplification the results were displayed using a mask of river boundaries.

Initially MaxEnt was executed as a "full model" with all 16 variables and 435 records. From the 16-variable model, two variables (average and minimum summer temperatures) were eliminated because they were highly correlated ($r > 0.8$) with other climate variables. The remaining variables were used to predict *M. margaritifera* distribution under two different scenarios, obtaining a final 12-variable model including four biotic variables (Table 3.2) and a final 9-variable model by excluding the biotic (host fish) variables (Table 3.2). The variable "Tertiary/Quaternary detrital deposits" was eliminated in the model including biotic variables, and "Granitic rocks" was removed in both models (the 9-variable and the 12-variable model), because they had zero contribution. Moreover, three additional models were developed by using the presence records of the three host fish species to evaluate the influence of the abiotic variables in predicting the distribution of the three host species; in each model (as with the mussel model) variables that had zero contribution were eliminated.

The models were evaluated by using the area under the receiver operating curve (AUC), parsimony and biological relevance (Elith *et al.*, 2006a). The AUC value is a measure of model performance that provides a threshold-independent relationship between the proportions of pixels correctly and incorrectly classified (Pearson *et al.*, 2007) and it can range from 0.5 (random prediction) to 1, thus the closer the value is to 1.0, the better the model is at differentiating between presences correctly classified and non-presences incorrectly classified (Pearson *et al.*, 2007, Phillips & Dudík, 2008). Finally, the logistic output of MaxEnt models was displayed in ArcMap. Probabilities of presence were calculated and masked by river boundaries. Comparison between the biotic versus no-biotic model predictions was made to evaluate the map predictions at a local scale.

Environmental predictor variables	Type	Units
(a) CLIMATE		
Average annual precipitation	*Continuous*	*mm*
Average summer precipitation	*Continuous*	*mm*
Average annual temperature	*Continuous*	*Celsius*
Average summer temperature	*Continuous*	*Celsius*
Maximun summer temperature	*Continuous*	*Celsius*
Minimum summer temperature	*Continuous*	*Celsius*
(b) HOST FISH		
Atlantic salmon density	*Continuous*	*Individuals $\cdot m^{-2}$*
Migratory trout density	*Continuous*	*Individuals m^{-2}*
Resident trout density	*Continuous*	*Individuals m^{-2}*
Salmonid biomass	*Continuous*	*$g \cdot m^{-2} \cdot year^{-1}$*
(c) LANDFORM		
Slope	*Continuous*	%
Forest Cover	*Categorical*	Binary
Elevation	*Continuous*	m
(d) GEOLOGY		
Granitic rocks	*Categorical*	Binary
Tertiary and Quaternary detrital deposits	*Categorical*	Binary
Metamorphic rocks	*Categorical*	Binary

Table 3.1.- List of abiotic and biotic predictor variables used in models for distribution and abundance of *M. margaritifera* in 20 river networks in northwest Spain. Data source: (a) Digital Climatic Atlas of the Iberian Peninsula (Ninyerola *et al.,* 2005), (b) Fish Database of European Streams (Beier *et al.,* 2007) and Xunta de Galicia, (c) Spatial Data Infrastructure of Galicia (SITGA) and CORINE Land Cover by the (EEA) and (d) (SITGA).

3.3 Results

The MaxEnt model for predicting probability of presence for *M. margaritifera*, including biotic factors for host fish, had an AUC of 0.938 with a standard deviation of 0.009. In this model, the range in number of parameters produced using the auto-features option of MaxEnt in different cross-validation runs were: raw (1 to 6 parameters), product (16 to 19), quadratic (0 to 1), reverse hinge (26 to 35), forward hinge (3 to 6), and threshold (47 to 64). The variables with important contributions were three biotic variables (salmonid biomass, resident trout density, Atlantic salmon density) and one abiotic variable (average summer precipitation) (Figures 3.2 and 3.3). The variable with the highest model improvement was salmonid biomass and the variable that decreased model gain the most when omitted was average summer precipitation. In contrast, when excluding biotic factors the MaxEnt for *M. margaritifera* presence had an AUC of 0.893 with standard deviation of 0.024. In this model average summer precipitation, elevation, average annual precipitation and maximum summer temperature (Figures 3.4 and 3.5) made larger contributions to predicting probability of mussel presence. The variable with the highest model improvement was average summer precipitation and the variable when omitted that decreased model gain the most was elevation.

The models with or without biotic variables differed in their spatial predictions of probability of mussel presence (Figure 3.6, 3.7, 3.8, 3.9 and 3.10). The map prediction differed in several areas (Figures 3.6 and 3.7). The model without biotic factors seems to over-predict probability of mussel presence; several locations where the species is known to be absent were predicted to have high probabilities (Figure 3.8, e.g. coastal small basins and dammed areas). Moreover, the partial dependence plots for each environmental predictor have similar shape for both models; however, they differ in the percent of contribution to the model (Figures 3.3 and 3.5).

Finally, the MaxEnt models executed separately for each of the three species of host fish showed the following AUC values: Atlantic salmon 0.973 and standard deviation 0.015, migratory trout 0.972 and 0.024 and resident trout 0.682 and 0.020.

For the two migratory hosts (Atlantic salmon and migratory trout) elevation was the variable that contributed the most whereas for resident trout the most important variable was detrital deposits. (Table 3.2 and Figure 3.11, see Annex III Figure III.1 for partial dependence plots for MaxEnt models of host fish presence).

Environmental predictor variables	Percent of contribution (a) Biotic	Percent of contribution (b) No Biotic
CLIMATE		
Average annual precipitation (mm)	**18.3**	**34.1**
Average summer precipitation (mm)	2.5	**10.8**
Average annual temperature (Celsius)	3.3	10.1
Maximun summer temperature (Celsius)	4.8	10.5
HOST FISH		
Atlantic salmon density (individuals $\cdot m^{-2}$)	**15.1**	-
Migratory trout density (individuals $\cdot m^{-2}$)	1.7	-
Resident trout density (individuals $\cdot m^{-2}$)	23	-
Salmonid biomass ($g \cdot m^{-2} \cdot year^{-1}$)	**23.2**	-
LANDFORM		
Slope (%)	1.5	5.8
Forest Cover (binary)	0.7	6.3
Elevation (m)	3.9	**20**
GEOLOGY		
Granitic rocks (binary)	0	0
Tertiary and Quaternary detrital deposits (binary)	0	0.1
Metamorphic rocks (binary)	1.9	2.2

Table 3.2.- List of abiotic and biotic predictor variables organized in categories and its estimates of the relative contribution to the models of probability of distribution of *M. margaritifera*. (a) model including biotic variables and (b) model excluding biotic variables.

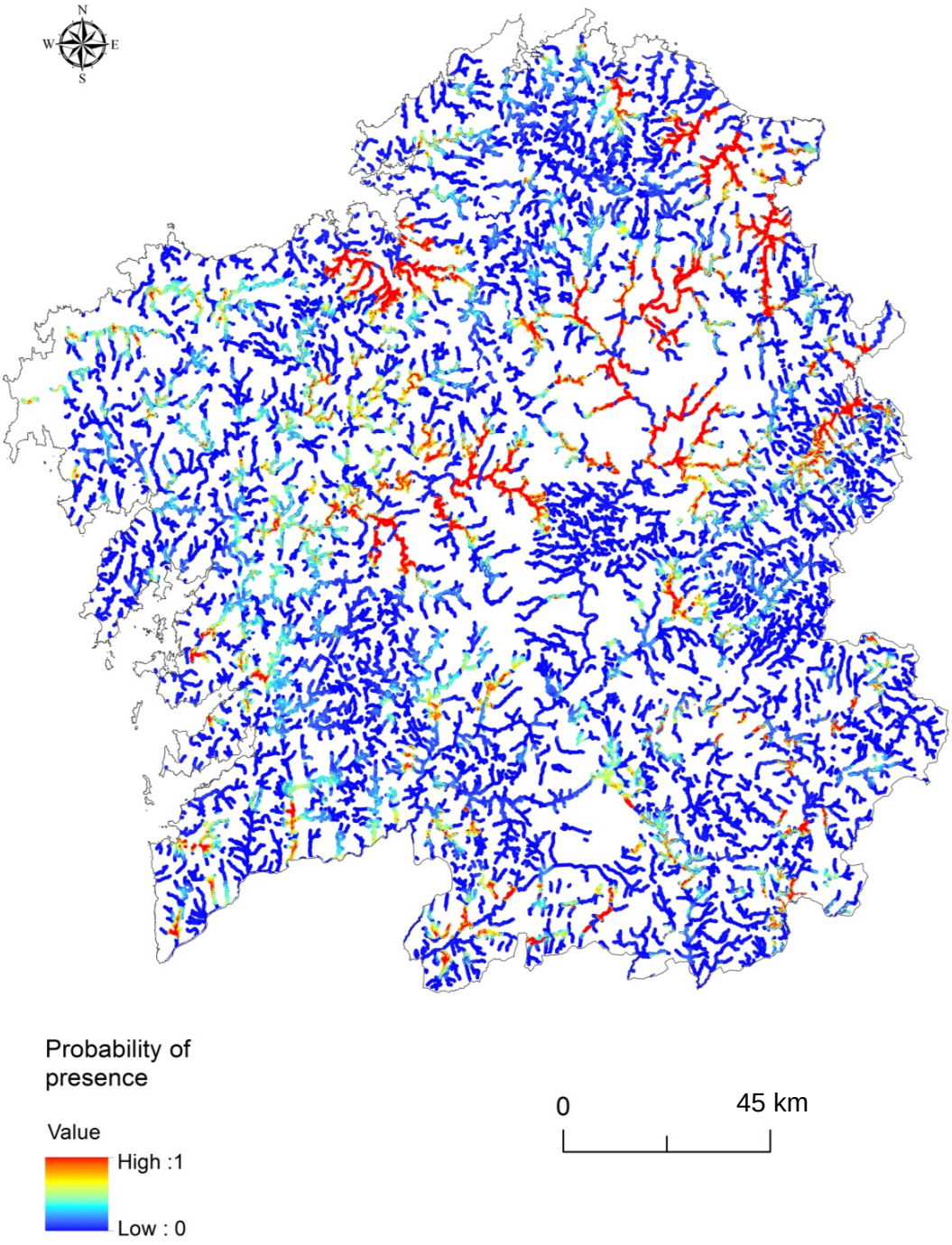


Figure 3.2.- Probability of presence of *M. margaritifera* in the study area predicted including biotic variables (logistic output).

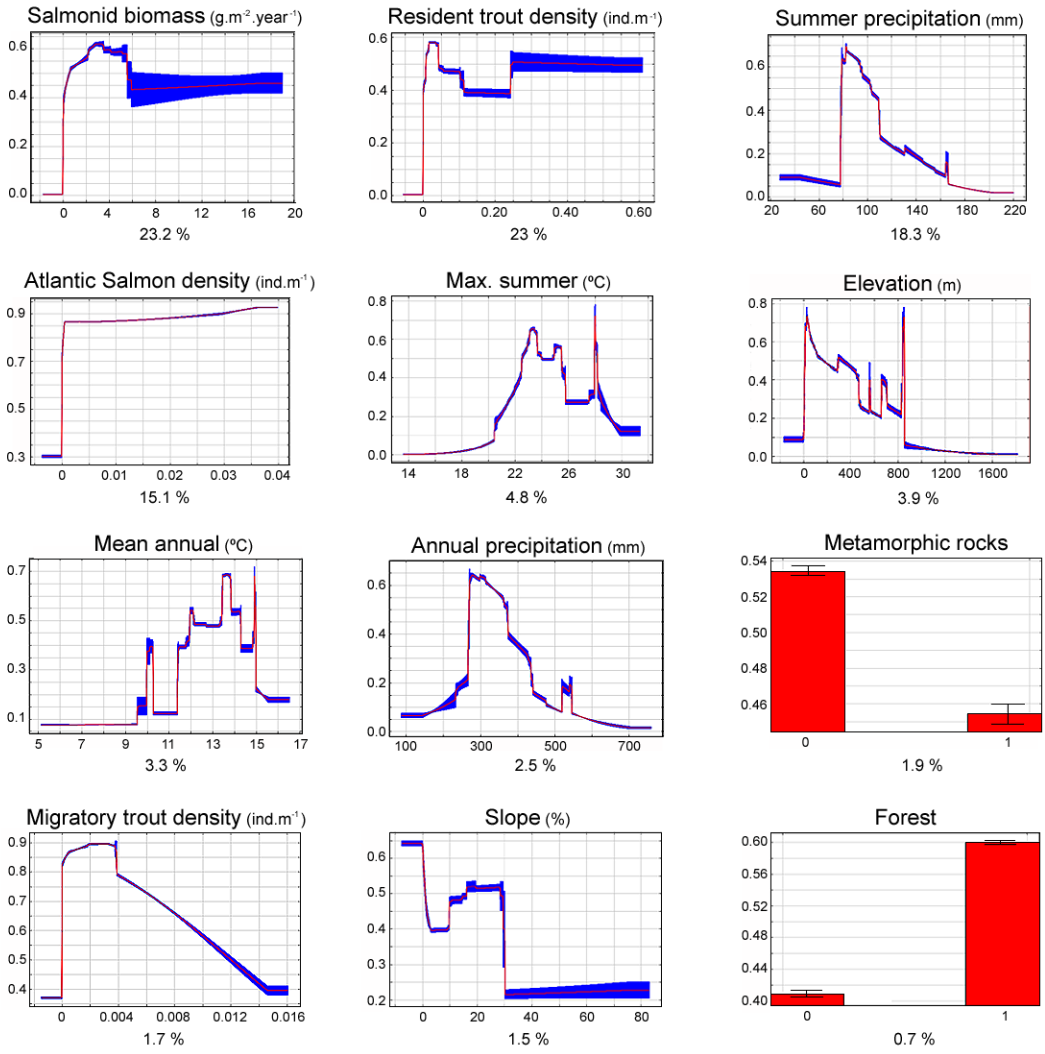


Figure 3.3.- Partial dependence plots of variables in MaxEnt model including biotic variables for predicting distribution of *M. margaritifera* in the entire study area. The percent contribution of each variable in the MaxEnt model is shown below each graph; the y-axis in each graph indicates probability of mussel presence (logistic output) as a function of the variable indicated. The curves show the mean response of the 10 replicate runs (red) and the mean +/- one standard deviation (blue, two shades for categorical variables).

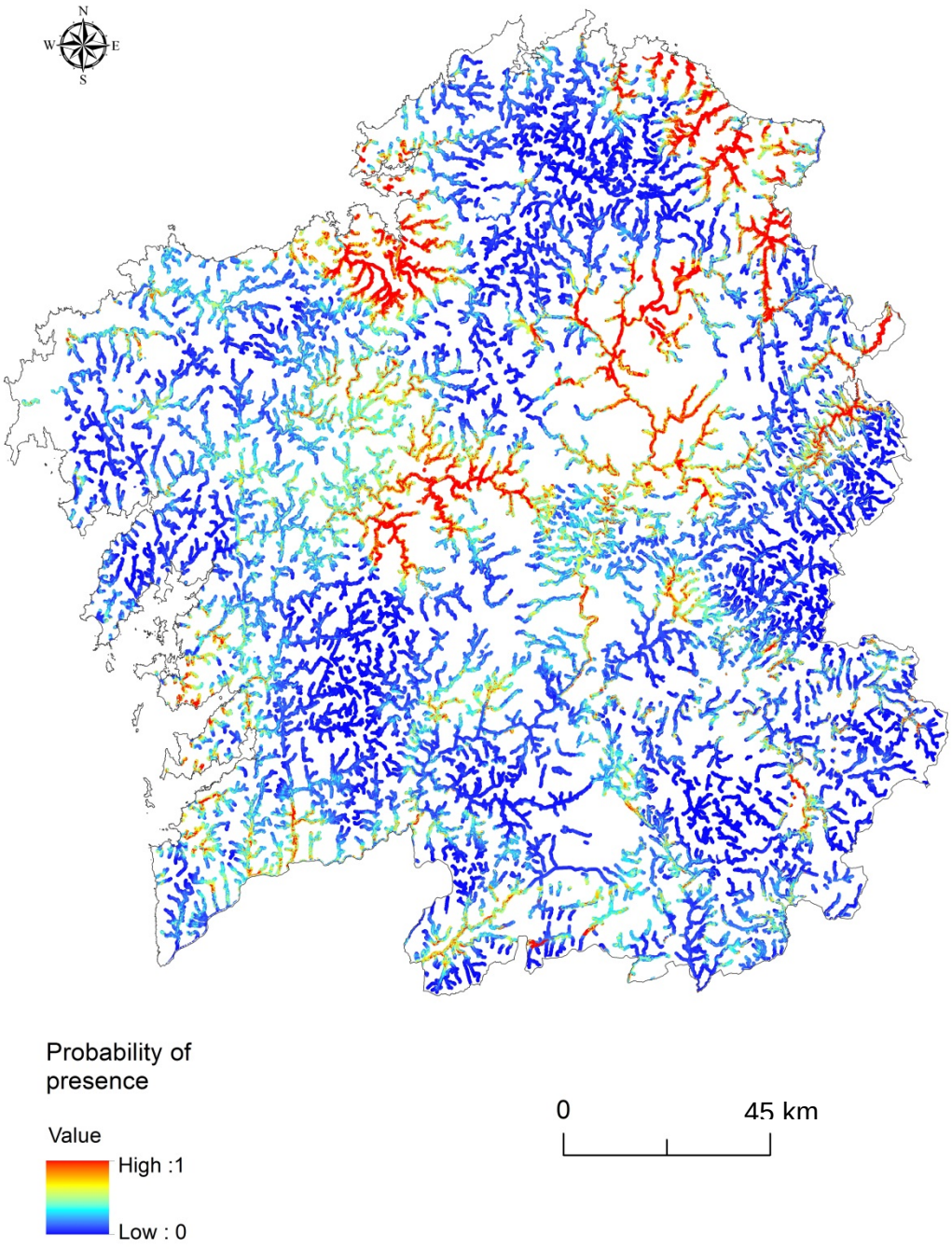


Figure 3.4.- Probability of presence of *M. margaritifera* in the study area predicted when excluding biotic variables (logistic output).

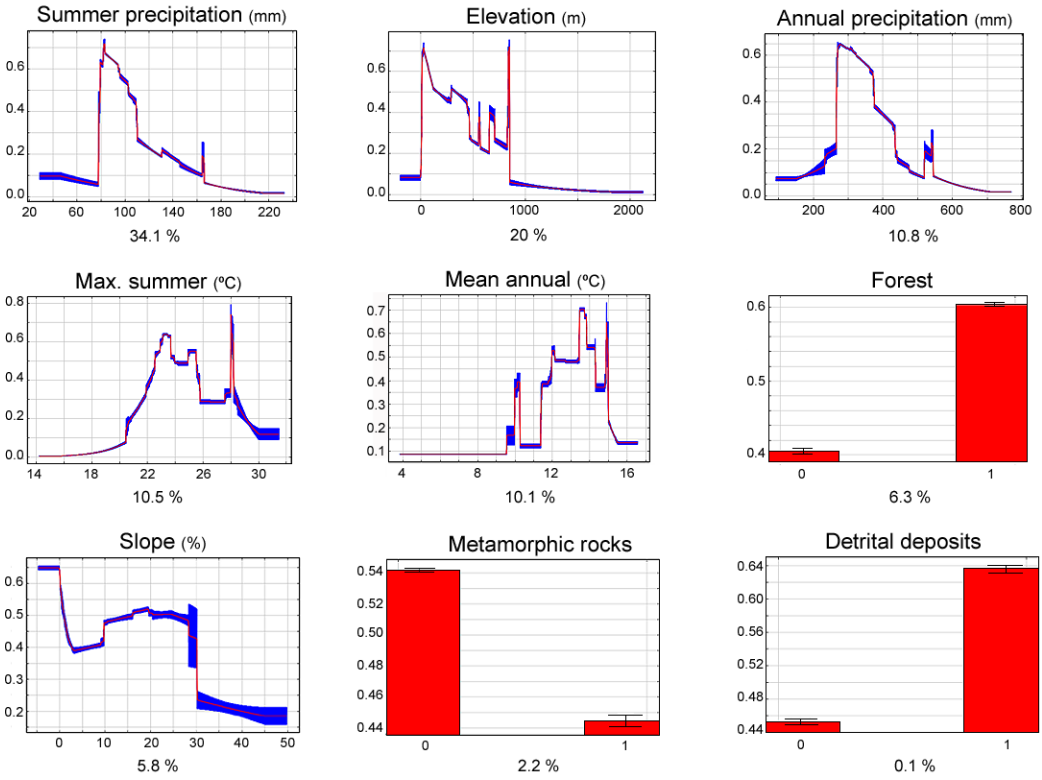


Figure 3.5.- Partial dependence plots of variables in MaxEnt model without biotic variables for predicting distribution of *M. margaritifera* in the entire study area. The percent contribution of each variable in the MaxEnt model is shown below each graph; the Y-axis in each graph indicates probability of mussel presence (logistic output) as a function of the variable indicated. The curves show the mean response of the 10 replicate runs (red) and the mean +/- one standard deviation (blue, two shades for categorical variables).

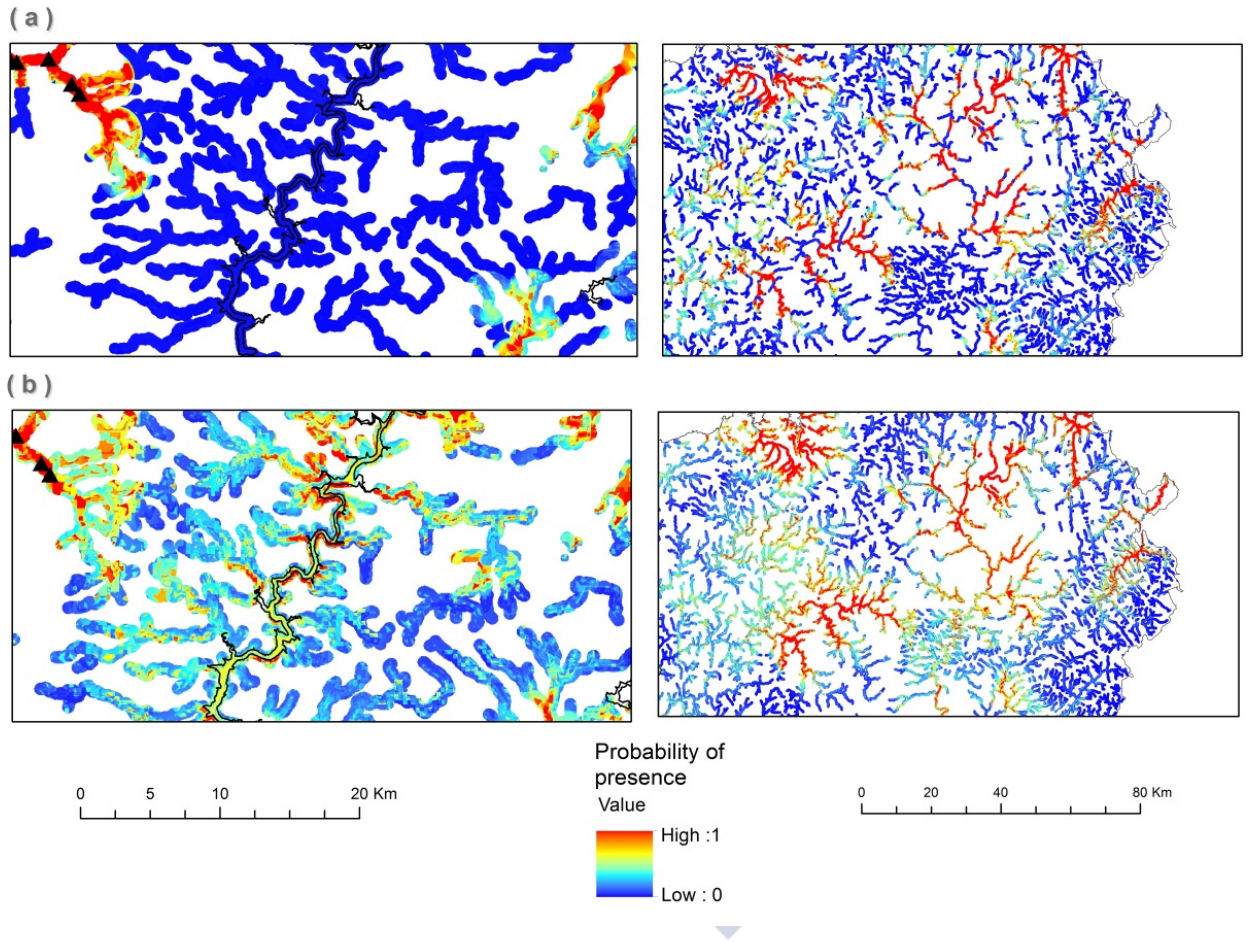


Figure 3.6.- Detail of the map predictions for *M. margaritifera* models in dammed areas of Miño basin (black lines denotes the current area occupied by reservoirs). Probability of presence of *M. margaritifera* in the study area predicted (a) model including biotic variables and (b) model excluding biotic variables.

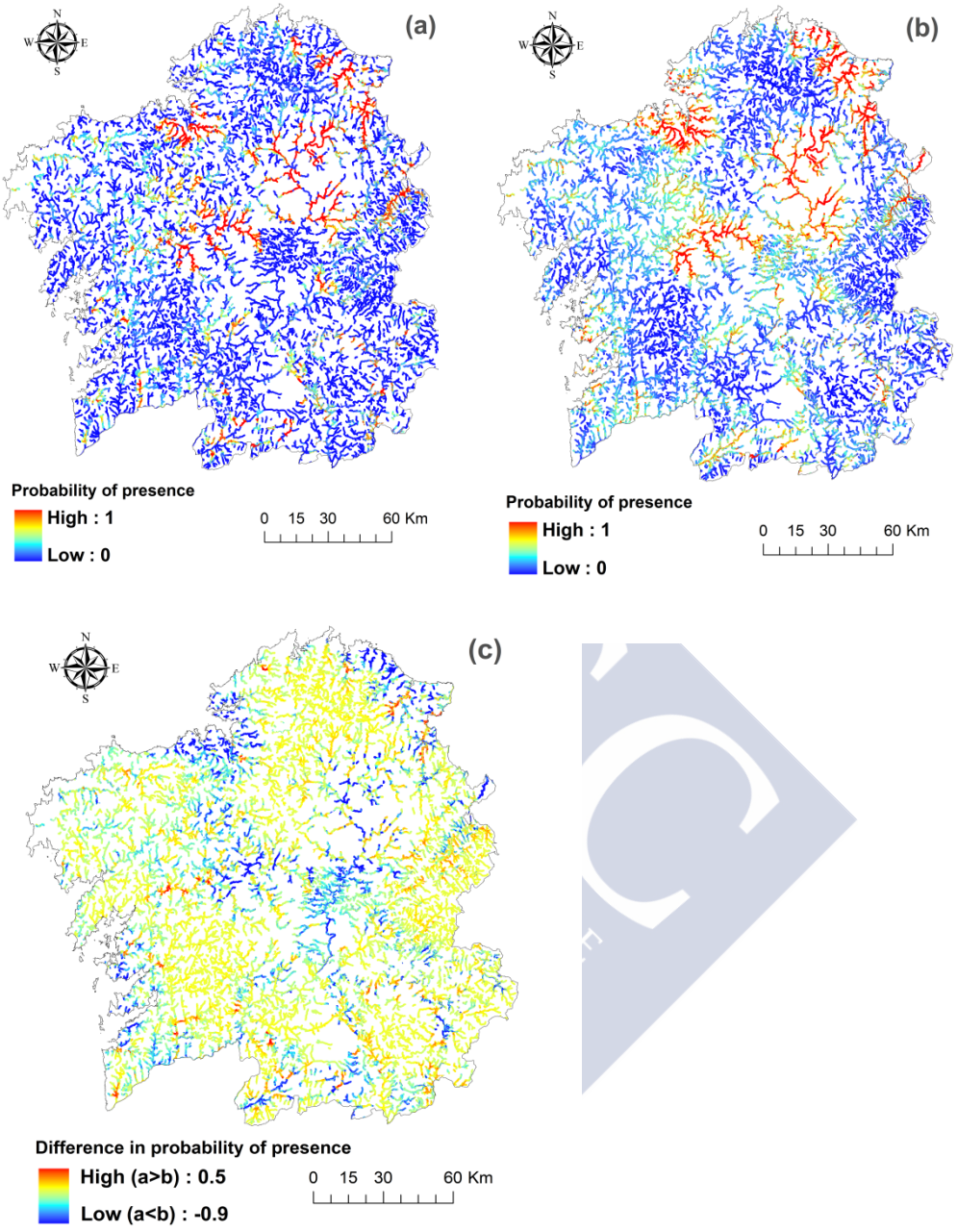


Figure 3.7.-Probability of presence of *M. margaritifera* in the study area predicted (a) including biotic predictor variables and (b) excluding biotic them. Panel (c) shows the difference (a minus b) in probability of presence for the two models; blue colours indicate areas where probability of presence is estimated to be higher when biotic variables are excluded. Orange to red colours show areas where including biotic interactions gave a higher probability of presence. Probabilities of mussel presence (logistic output) are shown in the three maps with the same colour scale.

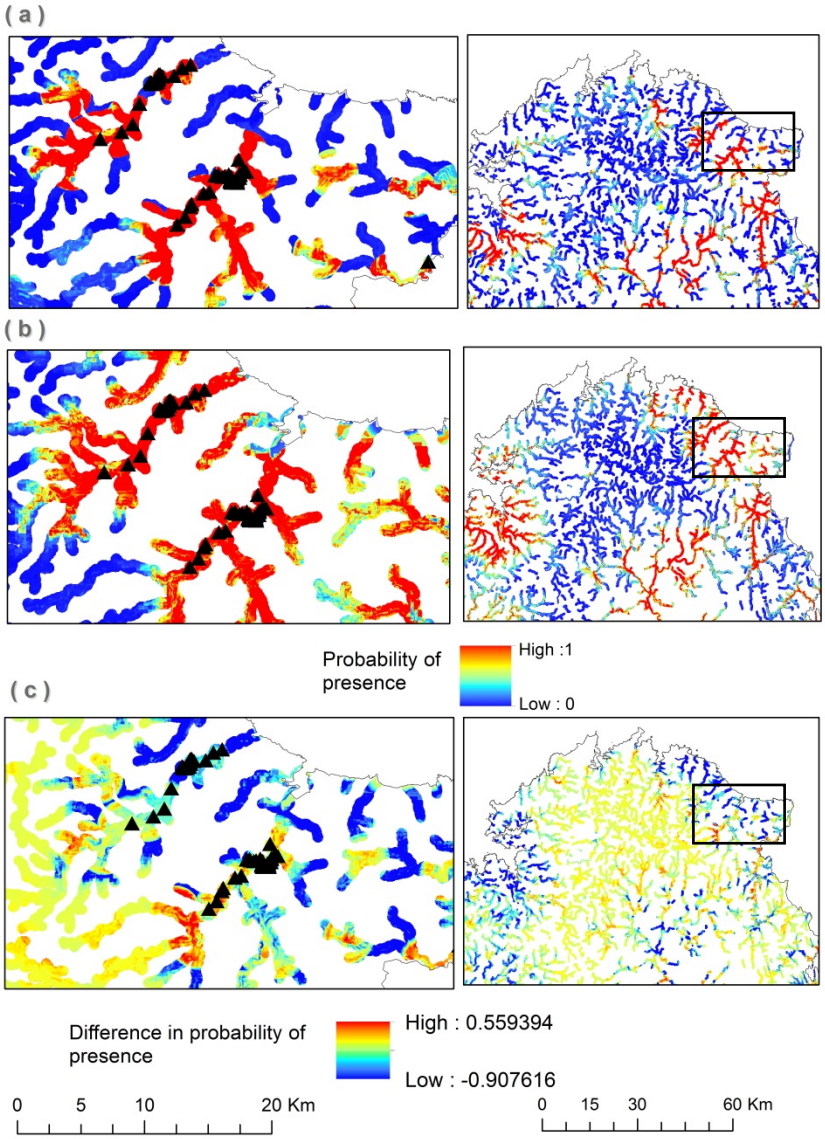


Figure 3.8.- Detailed maps (Ouro and Masma river basins) of probability of presence of *M. margaritifera* in the study area predicted (a) including biotic predictor variables and (b) excluding biotic variables. Panel (c) shows the difference (a minus b) in probability of presence for the two models; blue colours indicate areas where probability of presence is estimated to be higher when biotic variables are excluded. Orange to red colours show areas where including biotic interactions gave a higher probability of presence. Probabilities of mussel presence (logistic output) are shown in the three maps with the same colour scale and presence records of *M. margaritifera* are denoted by black triangles. Locator maps in the right column show the locations enlarged in the maps in the left column.

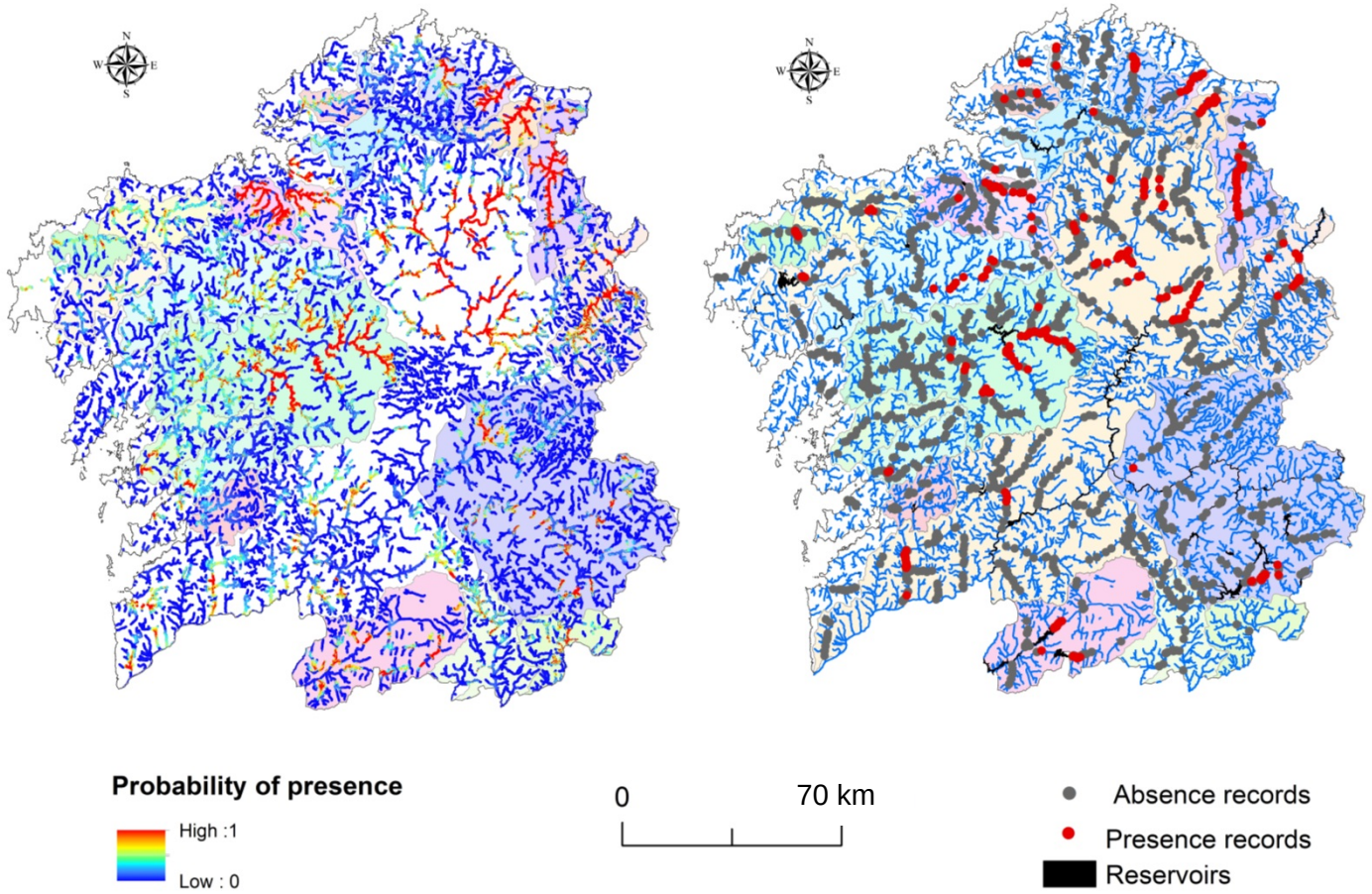


Figure 3.9.- Mapped predictions for probability of presence including biotic variables (left map) and current distribution of *M. margaritifera* in the study area (right map). Presence records of *M. margaritifera* are denoted by red dots and absence by grey dots.

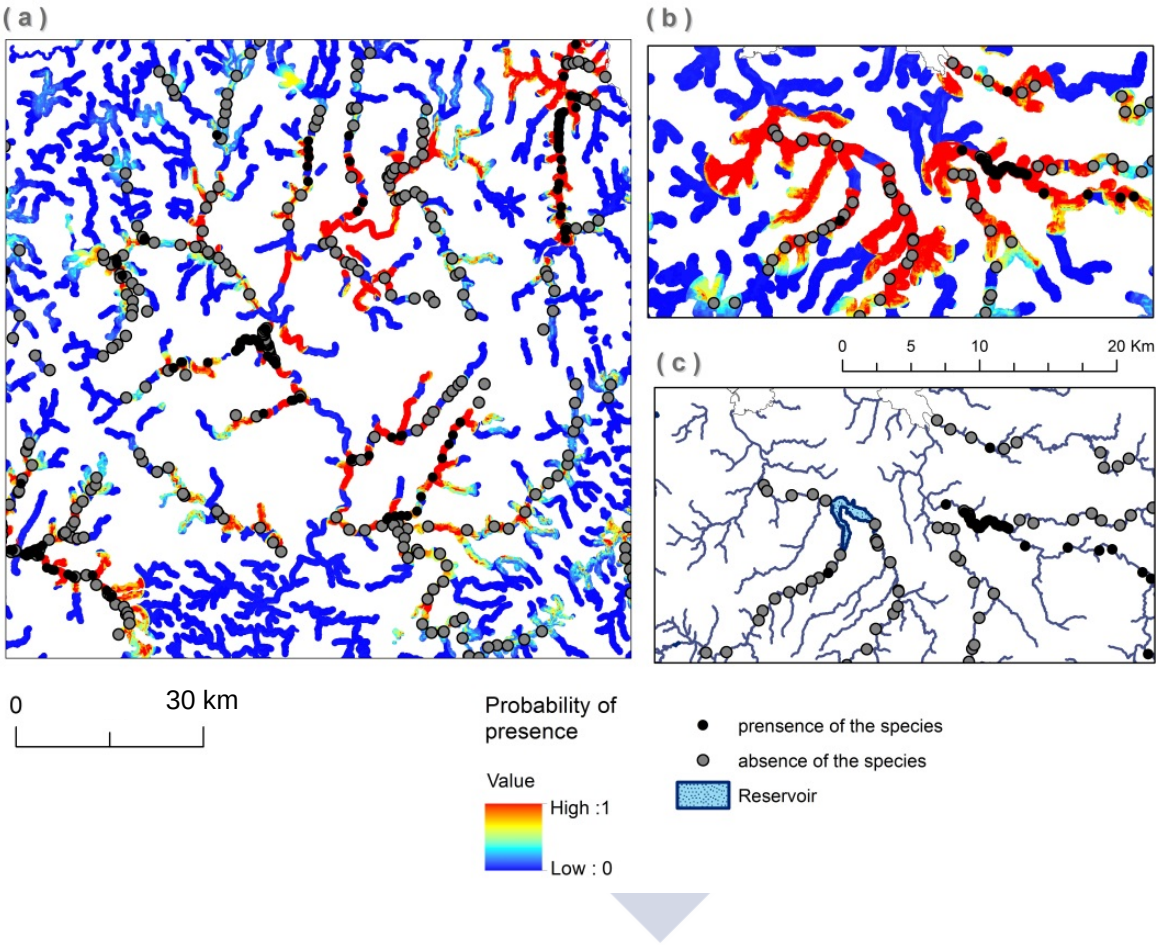


Figure 3.10.- Detail of mapped predictions for probability of presence including biotic variables of *M. margaritifera* for Upper Miño basin (a) and Mandeo and Mero basins (b). Red areas (high probability) show locations where further sampling is needed and absence points in areas with high probability of presence show areas that need future study to discern causes of absence. The current location of the reservoir in Mero basin is shown in map (c).

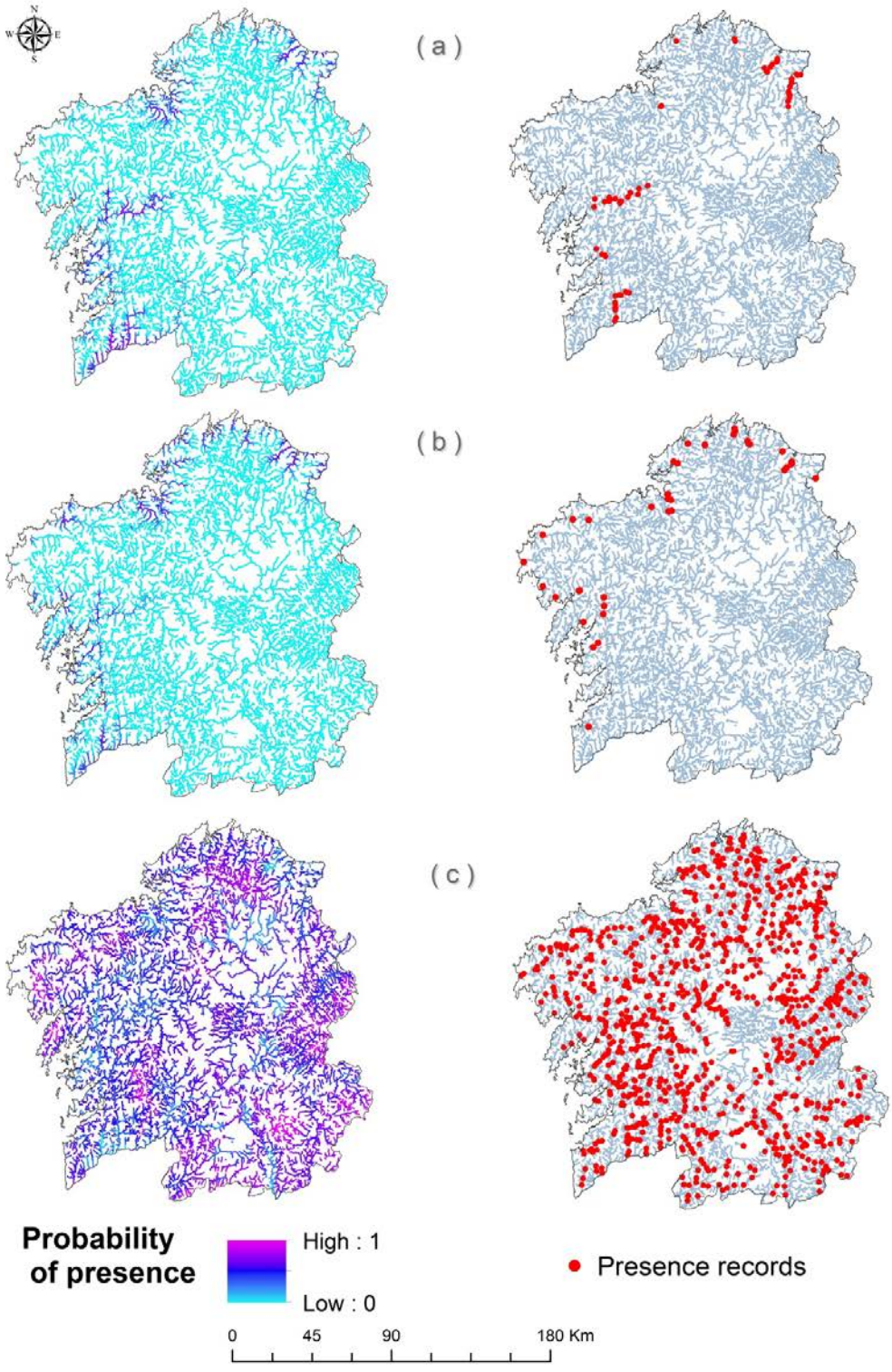


Figure 3.11.- MaxEnt probability of presence of the three host fish of *M. margaritifera* including abiotic predictor variables (logistic output) are shown in maps on the left side. Presence records used for develop the models are shown in maps on the right side. (a) Atlantic salmon (b) migratory trout and (c) resident trout.

3.4 Discussion

The results of this study illustrate a process for addressing complexity and biotic interactions when modelling distribution that is an important step in developing a conservation strategy for an endangered species. This case study shows a general way to use broad scale factors to predict distribution of an endangered species in multiple drainage basins. In spite of substantial spatial heterogeneity, natural environmental gradients in climate, discontinuities in geology and landform, host fish distribution and additional heterogeneity in human impacts across space and time, MaxEnt easily handled the complexity to predict probability of species presence.

This work shows that effects of biotic interactions are discernible at a regional scale in distribution of an endangered freshwater mussel species and are perceptible in the map predictions at a local scale (Figure 3.7). Furthermore, general ecological insights and management implications are gained for the endangered freshwater pearl mussel by using this approach.

Modelling Considerations

MaxEnt has been widely used to model species distribution since its introduction in 2004 (Elith *et al.,* 2011). In a comparative study it had the highest performance compared with 15 other algorithms (Elith *et al.*, 2006a). In our case study we obtained a MaxEnt model with an AUC of 0.938 with the 12 variables selected and the 435 presence records. Thus, the model showed an accurate prediction for the case study region by fitting the heterogeneity in this multiple river basin space with a large number of model parameters and it allowed discerning the effect of biotic interactions when predicting probability of species presence. This machine learning technique has been noted to allow complex relationships to be modeled (Elith *et al.,* 2011) and our results are consistent with this. An obvious prior limitation in using a machine-learning method was the difficulty in discerning ecological meaning from a high number of model parameters (Olden & Jackson, 2002). However, recent work specifically with MaxEnt has shown that under-parametrization is a more serious problem than

overparametrization (Warren & Seifert 2011). The choice of feature type in MaxEnt had negligible effects on predictive performance and Syfert *et al. (*2013) recommended the use of auto-features in MaxEnt applications. Moreover, the model parameters can be informative about the complexity of the study area, helping to identify sources of spatial heterogeneity.

The main objective in this chapter was to assess the influence of abiotic and biotic factors on species distribution in a complex landscape by using the current distribution of *M. margaritifera*. Furthermore, model results were also evaluated for the different host fish species by using the same abiotic predictors. Despite the aim of this study was not to develop a model for host fish species, the performance of the models for migratory fish was high (AUC of 0.973 for Atlantic salmon and AUC of 0.972 for migratory trout). In contrast resident trout substantially lower model performance (AUC of 0.682) compared with migratory hosts. This may be caused by the fact that resident trout are widespread in the study area and the species occupies a wide range of habitats and conditions (Figure 3.11c); however its abundance and biomass were important predictors for *M. margaritifera* distribution model (Figure 3.3). Moreover, it is necessary to acknowledge that additional factors not included in these analyses (e.g. human impacts, landscape modifications) affect distribution of an endangered species such as *M. margaritifera* and its hosts at smaller scales within rivers. For example in Europe, the declines of *M. margaritifera* have often been attributed to factors at a microhabitat scale, especially those affecting river bed dynamics such as sedimentation, increase in nutrients, substratum instability, or changes in chemical conditions, which affect the survival of the benthic juvenile stage (Bauer, 1988; Buddensiek *et al.*, 1993; Geist & Auerswald, 2007; Österling *et al.,* 2010). However, the machine-learning technique handled the complexity in the case study as shown with the AUC of 0.938. Moreover, by using broad-scale predictors and fine scale data of presence this study also provide meaningful information about ecology and conservation.

Ecological Insights

This work addresses the question "Do interactions between species affect distribution at regional scale? The results point out that effects of biotic interactions are discernible at a regional scale in distribution of an endangered freshwater mussel species. In this example biotic interactions are necessary for mussel populations to persist with successful reproduction and dispersal of mussels by parasitized fish is necessary for mussels to colonize habitat patches, which is crucial for metapopulation dynamics. Including biotic interactions in SDMs improved model AUC by about 4.5%. The map predictions were consistent generally with ecology of pearl mussels when biotic interactions with host fish are included (Strayer, 2008) and here, the biotic predictors made greater contribution in the SDM. Moreover, the map predictions when including biotic factors were improved at a local scale in many locations (Figures 3.6, 3.7, 3.8, 3.9, and 3.10).

The MaxEnt model prediction for *M. margaritifera* when including biotic factors fits with the general occurrence of filter-feeding invertebrates in river zonation (Vannote *et al.*, 1980) (Figure 3.2). The mussels were predicted to be absent in headwater streams and highest probability of presence was in 3rd order (Strahler, 1957) or higher order river segments where there is less temporal and spatial variation than in headwater reaches (Gomi *et al.*, 2002) (see Figure 3.2). Moreover, the mussels were predicted to occur in areas with low to intermediate values of slope (exceptions: rivers Eo and Navia) and in areas with lower to intermediate values of summer precipitation (except Tea and other small coastal basins). These combined influences of climate, geology and topography determine geomorphological processes influencing river channel dynamics (Montgomery, 1999) that may regulate the presence of specific habitat attributes for mussel habitats at finer spatial scales.

Thus, freshwater pearl mussel occurred in spatially limited areas within the study region where floodplains reaches of river corridor are generally in dynamic equilibrium with the flow regime (Nanson & Croke, 1992; Stanford & Ward, 1993; Ward *et al.*, 2002) providing a stable physical structure for mussel beds. Likewise

deposition processes that provide nutrients for the mussels govern these reaches. This may indicate that at a landscape scale the interactions between climate, geology and landform are key predictors that retain meaningful information about mussel habitat. The results of this chapter provide broad scale information about the importance of abiotic factors controlling the current distribution of *M. margaritifera* in the study area. As this study was carried out in the southern limit of its geographical range, it will help to develop further studies of climate change on *M. margaritifera* and its hosts.

Implications for Conservation

The procedures implemented can help a resource manager to understand sources of spatial variation in distribution of a species. Understanding complex spatial patterns is often required to manage biodiversity. Although this algorithm was proposed along with other modelling techniques as useful for identifying conservation areas for this species in Ireland (Wilson *et al.*, 2011b), that work differed from the work presented here (the modelling design did not included biotic interactions, it used a broader scale and different predictors). The findings gained with this study regarding conservation for *M. margaritifera* highlight the need to account for biotic interactions. The main findings are described below.

First, results for the auto-features MaxEnt model indicated salmonid production and density of resident trout were the most important variables for predicting mussel distribution; average summer precipitation, density of Atlantic salmon, and elevation were of secondary importance, with all other variables of minor importance. These results suggest that salmonid populations and summer precipitation are key factors controlling the distribution of *M. margaritifera* in the study area (Figure 3.3).

The performance of the models for migratory fish was high (AUC of 0.973 for Atlantic salmon and AUC of 0.972 for migratory trout) indicating that biotic variables used for predicting *M. margaritifera* distribution were also important for predicting migratory fish distribution. However, dams have fragmented most rivers in the study

area (Hervella & Caballero 1999, World Commission on Dams 2000) and they have excluded the Atlantic salmon and migratory trout from much of their former range in the northwest portion of the Iberian Peninsula (Hervella & Caballero 2002, Caballero *et al.,* 2006; García de Leaniz, 2008). It is noteworthy that the current distribution of *M. margaritifera* shown in this thesis closely matches the historical distribution of Atlantic salmon published by Hervella & Caballero (1999, 2002) in the study region. The models for migratory hosts in this thesis show that elevation is the most important variable for predicting distribution of the migratory hosts. As *M. margaritifera* is a long-lived organism its current distribution is maybe an indication of the past distribution of Atlantic salmon on the study area. In general, distribution and movement patterns of host fishes have been shown to influence the distributions of several freshwater mussel species including *M. margaritifera* (Watters, 1996; Vaughn, 1997; Haag & Warren, 1998). The results of this study show that this influence from host fish occurs within rivers and extends across river basins for *M. margaritifera* at regional scale.

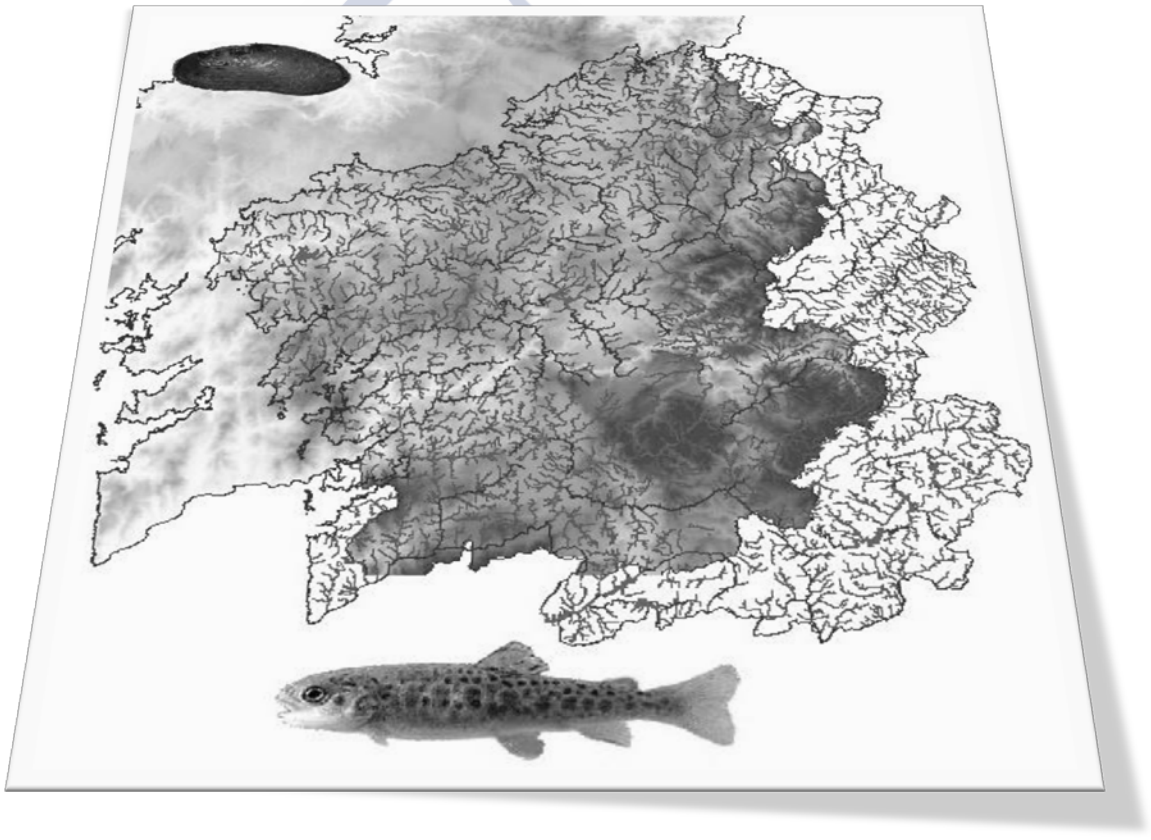
Second, because *M. margaritifera* is a long-lived species, its presence in rivers of the study area is indicative of suitable habitat in the past, even if those conditions are currently degraded from human impacts. Thus, the higher probabilities of species presence predicted by MaxEnt using broad scale variables are indicative of potential suitable habitat even when present day conditions may be unsuitable for reproduction or recruitment of the species.

Third, this study clearly indicates areas needing further research to quantify habitat quality in different zones and scales (Figures 3.9 and 3.10). The probability of presence provided by MaxEnt provides a useful fine scale spatial tool for assessing causes of species absence in habitat that is predicted to be suitable for the species (Figures 3.9 and 3.10). Gaining this kind of knowledge is necessary for local scale habitat restoration (Bond & Lake, 2003). Furthermore, new tributaries and river segments not sampled in this study were predicted to have higher probabilities of presence indicating new locations where further sampling efforts are need. Moreover, understanding how natural features and their interactions control mussel distribution

in different drainages and populations will help to design conservation units for mussels such as *M. margaritifera* and their hosts in different areas. Understanding ecological processes occurring in different areas will help to plan effective habitat restoration and management actions (Montalvo *et al.*, 1997).

Finally, there are extensive efforts underway in Europe and North America to recover *M. margaritifera* populations through captive breeding and habitat management (Gum *et al.*, 2011). These efforts will benefit from broad scale comprehensive studies to understand which factors are controlling mussel populations (Strayer, 2008), and having such knowledge will help to design integrative management actions for different areas. Moreover, as freshwater mussels are filter feeding bivalves that provide important ecosystem services in rivers (Spooner & Vaughn, 2008; Vaughn *et al.*, 2008) their conservation has important benefits for managing quality of freshwater and maintaining critical ecosystem functions.

4. MODELLING ABUNDANCE: RIVER ECOSYSTEM PROCESSES AND BIOTIC INTERACTIONS.



“The ups and downs of modelling”

”Non todo o que cae na rede é peixe”

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DE SANTIAGO
DE COMPOSTELA

4. MODELING ABUNDANCE: RIVER ECOSYSTEM PROCESSES AND BIOTIC INTERACTIONS

4.1 Introduction

Identifying factors that control or limit a species´ distribution and abundance is an important research area in ecology (Chesson, 2000) and a guiding concept in conservation biology (Lindenmayer & Hunter, 2010). As shown in the previous chapter, apart from the widely used abiotic factors generally called the bioclimatic envelope (Araújo & Peterson, 2012), inclusion of biotic interactions between species might improve species distribution models (SDMs) (Wisz *et al.,* 2013). In the previous chapter it also helped to retain important information of ecological and conservation concern. In this conext, it was pointed that in comparison with occurrence data, the use of abundance data when modelling might retain a higher amount of meaningful ecological information (Howard *et al.*, 2014).

In this study, understanding abundance patterns of freshwater riverine species such as *M. margaritifera* is a challenging issue because of its dependence on a host and because of the complexity of river environments. Rivers vary spatially and temporally in factors such as discharge, sediment load, dissolved solutes, nutrients, and temperature. Spatial location within a river system determines the general physical character of the river and its biota (Vannote *et al.,* 1980; Poff & Ward, 1990; Townsend, 1996; Allan & Johnson, 1997; Harris, 1998). Longitudinal position in a river affects the composition and extent of the adjacent riparian zone (Wissmar, 2004), the riparian contribution of allochthonous organic matter to the river ecosystem (Hynes, 1975; Elliott *et al.,* 1998), and the temporal interaction of the river with its floodplain (Ward & Stanford, 1995a; Hauer & Lorang, 2004). Even at a small local scale a river is typically heterogeneous with areas of sediment export or deposition and the degree of heterogeneity depends on spatial position in the drainage network (Vannote *et al.,* 1980; Ward & Stanford, 1995b). Many riverine species are patchily distributed at a local scale in response to variation in substrate composition, flow velocity, food availability, and other factors operating at multiple scales (Poff, 1997). Additional

intricacy in river ecosystems can arise with spatial or temporal variation in the interactions between species (for example between *M. margaritifera* and its hosts). Finally, consideration of human impacts to rivers worldwide adds another source of model complexity. Thus, spatial habitat heterogeneity, discontinuities, variable human-impacts, biodiversity, and temporal variation are all potential sources of model complexity in riverine systems.

In this Chapter a modelling process is described for the abundance of *M. margaritifera*, which accounts for biotic interactions and for the spatial complexity involved in riverine ecosystem processes. The modelling approaches include a non-spatial modelling technique (Path analysis) and a spatial modelling approach (Geostatistical mixed model). The objective of this work is to obtain meaningful information of ecological and conservation value for the freshwater pearl mussel.

4.2 Materials and Methods

Data and Environmental Predictor Variables

Analysis of species abundance was conducted with the predictor variables used in the previous chapter for modelling distribution (sixteen predictors variables belonging to four categories: Climate, land form, geology and host fish; see Table 3.1 and methods section of chapter 3 for a detailed description of each variable). This chapter analyses density of freshwater pearl mussel across in the study region using mussel density values estimated in Chapter 2 at the same 435 locations of mussel presence analysed in chapter 3. All analyses presented in this chapter used log-transformed mussel density and salmonids biomass because both statistical distributions on the original scale were highly skewed with long right tails.

Modelling Process of Species Abundance

The modelling approaches described below (non-spatial model and spatial model) were applied to the full dataset. However, the distribution model results described in chapter 3 (high number of model parameters) suggested possible spatial

heterogeneity in the study region. Thus, the full data set was divided in domains (strata or subsets) to explore potentially meaningful sources of spatial heterogeneity. Two domains regarding host fish were used based upon two reasons. First, there is a clear river fragmentation by dams in the study region that conditions the distribution of the migratory host fish. MaxEnt distribution modelling (see discussion of Chapter 3) should indicate complex relationships between mussel presence and host fish suggesting spatial heterogeneity regarding host fish. Therefore, I analize mussel abundance for two subsets of data, representing presence or absence of migratory host fish. The first subset was composed of 274 records where migratory salmonids were absent and the other contained 161 records where one or both migratory host fish were present. This stratification was conducted by using a non-spatial model (Path analysis) and a spatial model based on river networks. For the path analysis approach several hypotheses were tested about sources of spatial heterogeneity that can affect mussel abundance. The different domains explored helped identify sources of spatial heterogeneity, and those analysis results are shown in Annex IV.

Non-Spatial Model

Path analysis (Wright, 1934) was used to model species abundance. It is a technique for decomposing correlation coefficients into direct and indirect parts and it is equivalent to a multiple regression in standardized correlation form (Johnson & Wichern, 1982; Lynch & Walsh, 2000) the way the method was used here. Thus, the path models have simple structure and they include a residual term. The solution for path coefficients (direct effects) in a regression model is simple and straightforward. In matrix notation, the vector of correlations between a response variable, in this case mussel density, and the predictor variables ($\mathbf{r}_{zy}$) can be written as the product of the correlation matrix for the predictor variables ($\mathbf{R}_{zz}$) and a vector of path coefficients ($\mathbf{p}_y$), i.e.

$$\mathbf{r}_{zy} = \mathbf{R}_{zz}\mathbf{p}_y$$

This system of simultaneous equations involving the observed correlations and path coefficients can be solved to obtain the vector of path coefficients by

$$\mathbf{p}_y = \mathbf{R}_{zz}^{-1}\mathbf{r}_{zy}$$

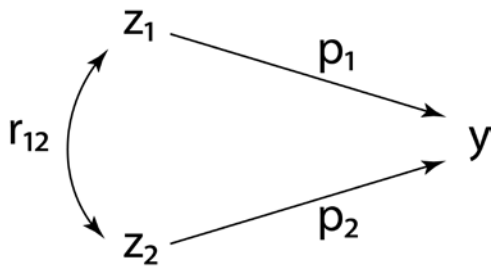
where the exponent denotes matrix inversion.

The residual error variance in a multiple regression path model is given by

$$\mathbf{p}_{Ye}^2 = 1 - \mathbf{r}_{zy}'\mathbf{R}_{zz}^{-1}\mathbf{r}_{zy}$$

where the prime denotes transposition. The contribution to the variance of the standardized response variable (variance=1) that is made by a predictor variable's direct contribution is represented by the square of its path coefficient. Indirect contributions of each variable through a second variable are represented by the product of the correlation coefficient between the variables and the path coefficient of the second variable. Total indirect effects for each variable were calculated as the sum of the individual indirect effects.

To illustrate the total effect of a variable, consider the example below for three variables. Here, p_1 and p_2 denote the direct effects of each predictor variable on the dependent variable (y) and r_{12} is the correlation between the predictor variables. The indirect effect of z_2 on y is p_1r_{12} and the total effect of z_2 is $p_2 + p_1r_{12}$. If p_1, p_2, and r_{12} are all of the same sign, then indirect effects are reinforcing and the total effect is larger. If there is a difference in sign, then the indirect effect offsets the direct effect.



The path models analysed were standard partial mutiple regressions of mussel density on predictors. Path analysis decomposed correlations between predictor variables and mussel density into direct and indirect effects. In path analyses, mussel density was the dependent variable and the abiotic and biotic interaction factors were the predictor variables. Two variables (mussel density, salmonids biomass) were transformed to natural logarithms prior to analysis, data for each variable were standardized and product-moment correlation coefficients were obtained. Path analysis were conducted in the SAS-IML language (SAS Institute, Inc. 2010) and applied to the full dataset of 435 records and to the different subsets (see Annex IV for extra subsets analysed).

This study did not intend to infer causality in the models but rather it used the method to gain a better intuitive appreciation of 1) the direct effects of predictors individually and by category of variable (climate, geology, landform, and host fish), and, 2) the indirect effects of predictor variables that occur through their correlations with other variables. Results for path analyses are presented in a new way to emphasize (a) the relative strength of direct effects using varying arrow widths and (b) the relative importance of total direct effects for each category of variables (climate, geology, landform and host fish) with differential shading of quadrants in the path model figures. The graphical summary of each path analysis is combined with a map showing the basins included in the each domain or strata.

Spatial Model

The modelling techniques developed by Peterson and Ver Hoef (2010, 2014) were applied to analyse abundance data in the framework of spatial models for river ecosystems. In this context, a river is a dendritic ecological network (Peterson *et al.*, 2013) with headwaters, accumulating directional flow and an outlet (Figure 4.1). A river lies within a drainage basin, which contributes water and materials to the river; thus, the drainage basin is a fundamental unit in spatial river ecology. In rivers, flow is an important endogenous process, as are organismal movements upstream against the flow, along with exogenous processes that contribute materials from the landscape of

the drainage basin. These spatial models account for the processes that occur in river ecosystems: the spatial configuration, the longitudinal connectivity, the discharge, and the flow direction in a stream network (Peterson & Ver Hoef, 2010).

The principles of these analytical techniques are that; first, they have valid (positive semi-definite) covariance matrices based on different hydrological relationships (Cressie *et al.,* 2006; Ver Hoef *et al.,* 2006; Peterson *et al.,* 2013) allowing complex relationships to be modelled and, second, they use a variance component approach allowing a mixture of autocovariance models (Euclidean and stream models) to be incorporated into a single geostatistical model. The geostatistical modelling approach for stream networks was applied for the analyses of abundance data of *M. margaritifera*.

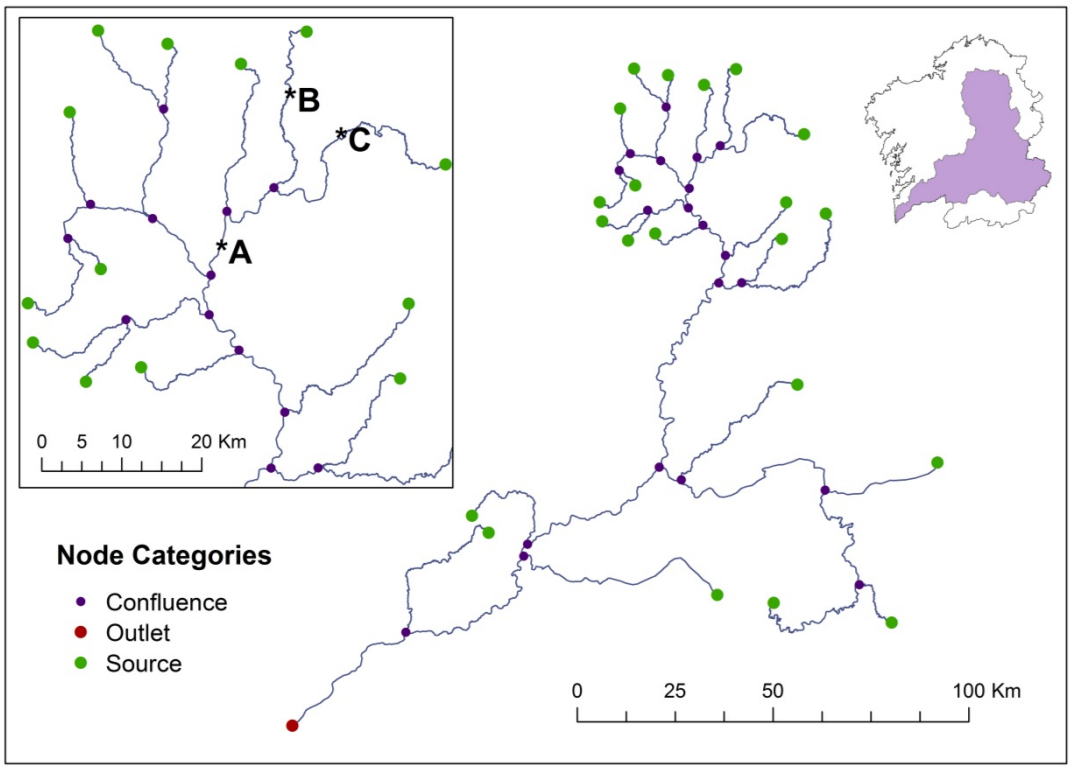


Figure 4.1.- Detail of the stream network of the Miño basin. The upper-right map shows the location of the Miño drainage basin in the study area (Galicia). In the legend the different node categories are displayed. The upper-left map shows a detailed map of the Miño stream network where A, B and C represent sites for mussel abundance. A is flow-connected with B and C while B and C are flow-unconnected sites.

Analyses of mussel abundance were conducted with routines implemented in the SSN package (Ver Hoef *et al.,* 2006; Peterson & Ver Hoef, 2010; Ver Hoef & Peterson, 2010; Ver Hoef *et al.,* 2014) in R (R Development Core Team, 2010). To accomplish the analyses, spatial stream networks were constructed with ArcGIS using FLoWs and STARS toolsets (Peterson & Ver Hoef, 2014). Watershed area was used to calculate spatial weights. The spatial data of this study included 20 networks and the same fixed effect environmental covariates used in species distribution modelling. The same procedures described below were used for the full data set and for two subsets of sites representing presence or absence of migratory host fish.

Moving average functions for spatial covariance in river networks (Peterson & Ver Hoef, 2010) implemented in the SSN package were evaluated in the spatial models (tail-up, tail-down and Euclidean). Occurrence of spatial autocorrelation in the study data was evaluated visually with Torgegrams (Peterson *et al.,* 2013; Ver Hoef *et al.,* 2014), which are semivariograms depicting semivariance for flow-connected and flow-unconnected sites as a function of stream distance separating pairs of sites. Estimates of partial sill and geostatistical range for the different models of spatial autocorrelation were obtained and different models were compared with the Akaike information criterion (AIC) (Akaike, 1973) to guide subsequent modelling decisions. The best model was identified as having the lowest AIC value.

Choice of predictor variables to include in the model of mussel abundance was made by assessing significance first with a non-spatial multiple regression model. Subsequently I evaluated the same full set of predictor variables in a spatial linear mixed model that included three models of spatial autocorrelation, exponential tail-up, exponential tail-down, and Euclidean components. Maximum likelihood estimation was used for model fitting and predictor variable selection. In this case the contribution of the Euclidean component was negligible and the comparison of AIC including and excluding the Euclidean autocovariance function indicated that excluding it yielded a better model. Residuals from a spatial linear mixed model including exponential tail-up and tail-down sources of spatial autocorrelation were examined,

the outliers for mussel abundance were removed, and the spatial mixed model was refitted to the screened data. Significant predictor variables were retained and the spatial linear mixed model was refitted iteratively with maximum likelihood estimation, dropping non-significant covariates from the model. The AIC was used to choose the final set of fixed effects in the mixed model.

Because there have been few analyses published using the spatial autocorrelation models in the SSN package (Isaak *et al.*, 2014), I evaluated in this study all 25 pairwise combinations of the five (linear with sill, spherical, exponential, Mariah and Epanechnikov) (Ver Hoef *et al.,* 2006; Garreta *et al.,* 2010) tail-up and tail-down functions, respectively, to identify appropriate models of spatial autocorrelation for the mussel abundance data in this study. For each mixed model the same set of previously identified fixed effect predictor variables were included along with the random effects for the tail-up and tail-down models. For model selection, restricted maximum likelihood estimation was used. The AIC was computed for each model and the best models were identified as having the lowest AIC value. For each of the best spatial linear mixed models, bias was also inspected along with root mean square prediction error and standardized mean square prediction error. Generalized R^2 was obtained for the fixed effect predictor variables and variance components were obtained for the random effects. The relative fractions of variance explained in mussel abundance by the fixed and random effects were calculated.

4.3 Results

Non-Spatial Model

Path analysis was conducted on mussel abundance with the predictor variables used in distribution modelling in the third chapter of this thesis. Across the entire study region (N = 435) the predictors explained 18.2% of the variation in density of *M. margaritifera* (Figure 4.2 and Table 4.1). Direct effects of climate (darker shading in figures) were more important than landform, host fish, and geology. Mean annual temperature and elevation were the largest direct effects but both variables had

indirect effects opposite in sign so that the total effects of those variables were near zero (Table 4.1).

Path analyses showed nearly a two-fold difference in the amount of variance explained for mussel density depending on the presence or absence of migratory salmonids. For records where the migratory host fish were absent, only 15.1% of the variance in mussel density was explained (Figure 4.3). In contrast, 28.2% of the variance in density was explained for records where migratory host fish were present (Figure 4.4). In the absence of migratory host fishes, climate and landform predictors had larger direct effects (darker quadrant shading) than host fish or geology (Figure 4.3). With presence of migratory host fish, direct effects for landform were most important followed by host fish, geology, and climate (Figure 4.4).

Regarding positive direct effects in descending order of importance for the analysis of records with presence of migratory host fishes were: elevation, anadromous migratory trout, metamorphic rock, and Atlantic salmon. More negative direct effects were obtained for forest cover, annual precipitation, detrital rocks, and slope (Fig. 4.4). Path analysis also provided additional information about indirect effects of variables that arise through their correlations with other variables. Stronger indirect effects (Table 4.1) were positive for salmonids biomass, slope, and metamorphic rocks and negative for summer precipitation, mean annual temperature, maximum summer temperature, and granitic rock. Total effects of variables were stronger and positive for metamorphic rock, elevation, and salmonid biomass and negative for annual and summer precipitation, detrital rocks, mean annual temperature, and granitic rock. The results for the other domains or strata conducted by using path analyses are show in Annex IV.

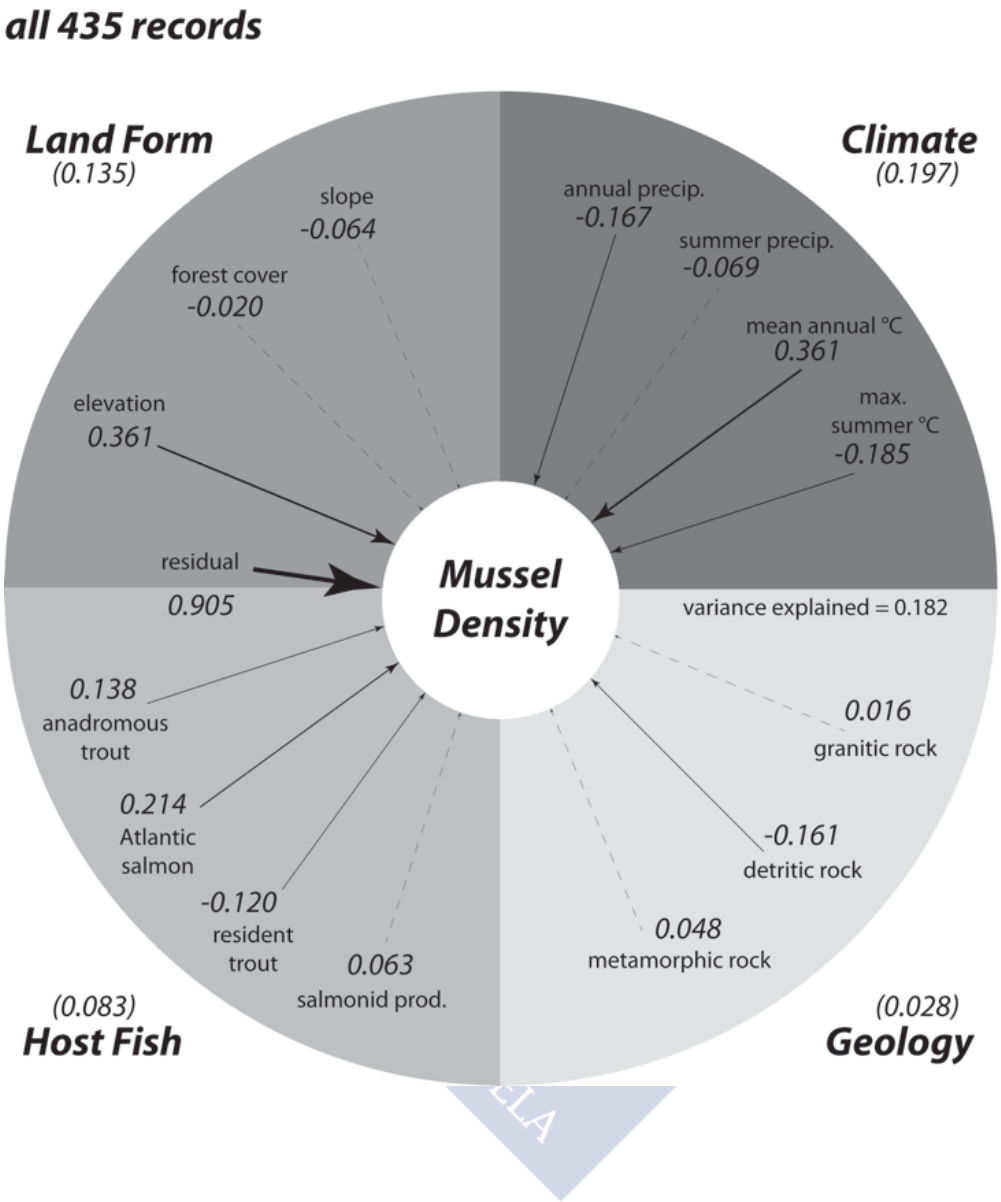


Figure 4.2.- Path analysis results for *M. margaritifera* density data for the full dataset of 435 records. For simplicity path analysis results are displayed in this figure where arrow width is proportional to the size of the direct effect of each variable and the degree of shading in the quadrants denotes relative importance of the sum of squared direct effects for the four categories of predictors. To further simplify the figure, correlations between the predictor variables are omitted. The variable salmonid biomass is represented in this graph by the name salmonid production (salmonid prod.).

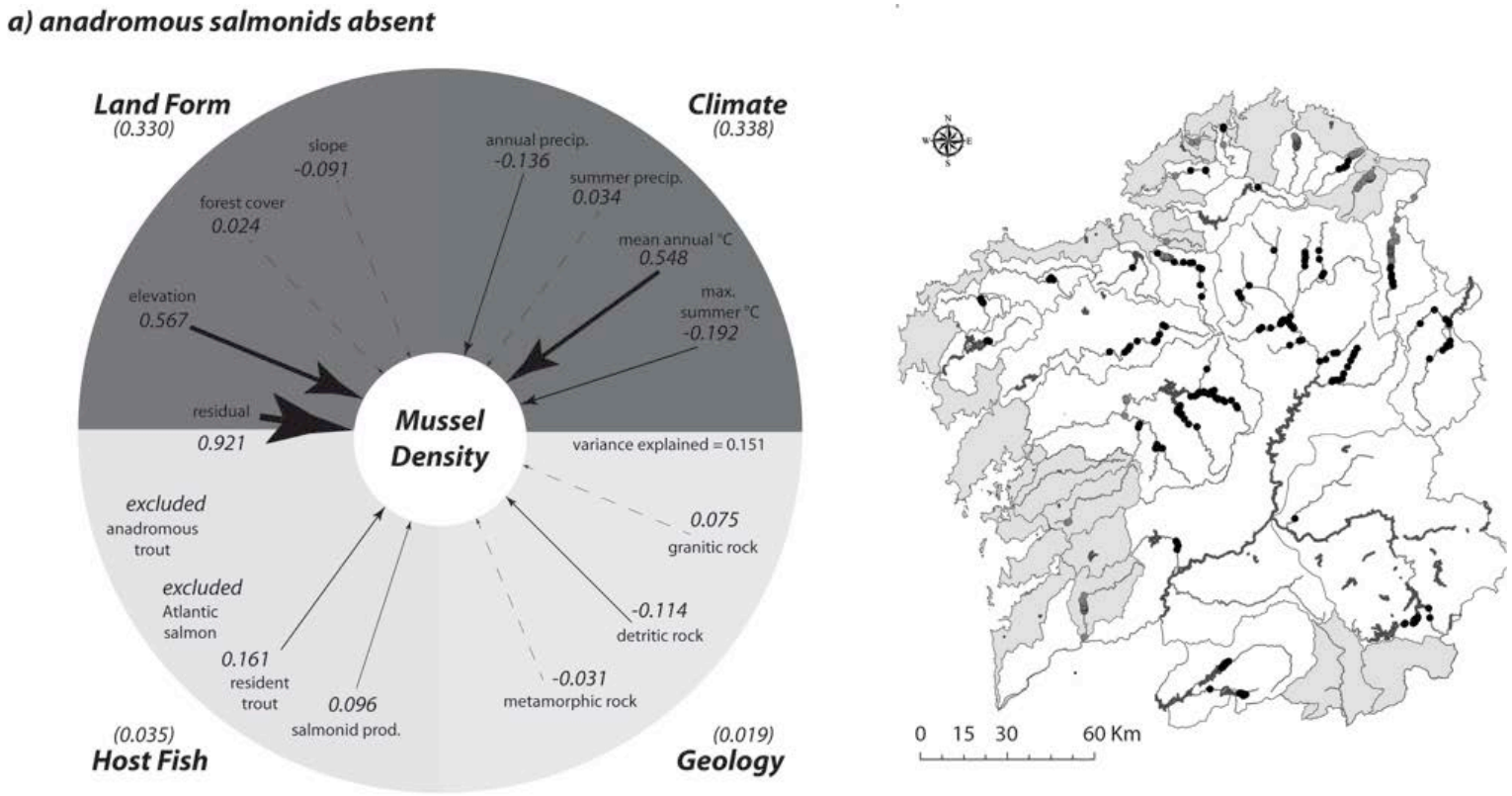


Figure 4.3.- Path analysis (left) for *M. margaritifera* density for the subset of anadromous salmonids absent and the map (right) depicting 274 sites and river basins included in the analysis (white background). Arrow width is proportional to the size of the direct effect of each variable. Degree of shading in the quadrants denotes relative importance of the sum of squared direct effects for the four categories of predictors. Records included in an analysis are shown as black dots in the map whereas those records within a basin not included in the analysis are shown as grey dots. The variable salmonid biomass is represented in this graph by the name salmonid production (salmonid prod.).

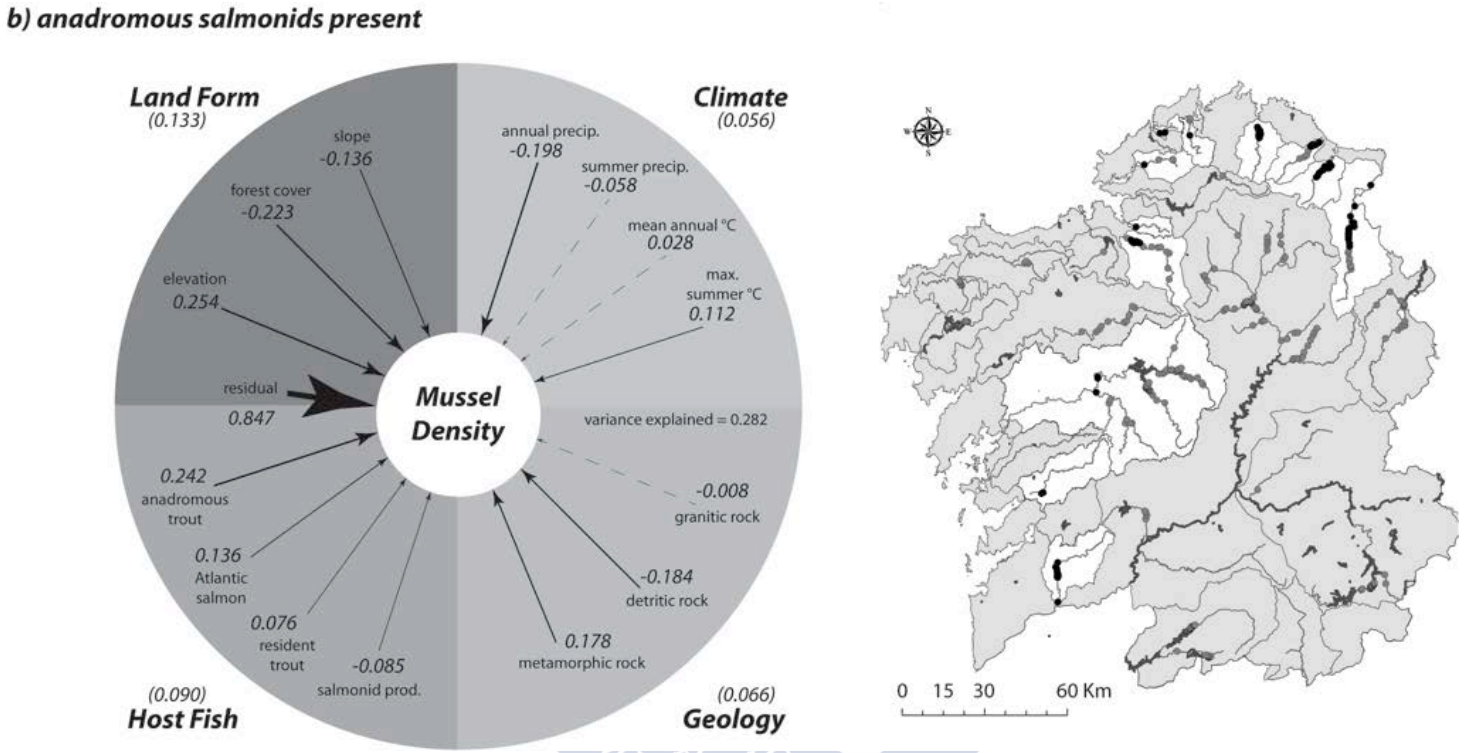


Figure 4.4.- Path analysis (left) for *M. margaritifera* density for the subset of anadromous salmonids present and the map (right) depicting 161 sites and river basins included in the analysis (white background). Arrow width is proportional to the size of the direct effect of each variable. Degree of shading in the quadrants denotes relative importance of the sum of squared direct effects for the four categories of predictors. Records included in an analysis are shown as black dots in the map whereas those records within a basin not included in the analysis are shown as grey dots. The variable salmonid biomass is represented in this graph by the name salmonid production (salmonid prod.).

Variable	Indirect	Total Effect
All 435 abundance records (Figure 4.2)		
detrital rocks	-0.029	-0.190
metamorphic rocks	0.099	0.147
granitic rocks	0.025	0.041
elevation	-0.369	-0.008
forest cover	0.055	0.035
slope	0.211	0.147
salmonid biomass	-0.005	0.058
anadromous trout	0.031	0.169
Atlantic salmon	-0.091	0.123
resident trout	0.032	0.088
annual precipitation	-0.099	-0.266
summer precipitation	-0.203	-0.272
mean annual temperature	-0.302	0.059
maximum summer temperature	0.140	-0.045
Anadromous salmonids present (Figure 4.4)		
detrital rocks	-0.069	-0.253
metamorphic rocks	0.184	0.362
granitic rocks	-0.151	-0.159
elevation	-0.054	0.200
forest cover	0.085	-0.138
slope	0.253	0.117
salmonid biomass	0.261	0.176
anadromous trout	-0.105	0.137
Atlantic salmon	-0.061	0.075
resident trout	0.010	0.086
annual precipitation	-0.133	-0.331
summer precipitation	-0.272	-0.330
mean annual temperature	-0.235	-0.207
maximum summer temperature	-0.197	-0.085

Anadromous salmonids absent (Figure 4.3)

detrital rock	-0.062	-0.176
metamorphic rock	-0.036	-0.067
granitic rock	0.140	0.215
elevation	-0.343	0.224
forest cover	-0.089	-0.065
slope	0.144	0.053
salmonid biomass	-0.180	-0.084
anadromous trout	---	---
Atlantic salmon	---	---
resident trout	-0.037	0.124
annual precipitation	-0.088	-0.224
summer precipitation	-0.260	-0.226
mean annual temperature	-0.630	-0.082
maximum summer temperature	0.243	0.051

Table 4.1.- Indirect and total effects of each variable in the path models; direct effects of each variable are shown in Figures 4.2, 4.3 and 4.4.

Spatial Model

Initially a non-spatial multiple regression model to the full data set was fitted. Important biotic variables in this model included resident trout (P = 0.042), migratory trout (P = 0.015), and Atlantic salmon (P = 0.001) along with abiotic variables elevation (P = 0.016), average annual temperature (P = 0.018), and maximum summer temperature (P = 0.019) (Table 4.2). I subsequently evaluated spatial mixed models that can account for spatial autocorrelation.

The Torgegrams (Figure 4.5) and geostatistical range estimations indicated spatial autocorrelation in mussel abundance occurred to a distance of aproximately 17 km, calling into question the significance values in the non-spatial multiple regression model. Therefore, an exploratory spatial mixed model was fitted using the same set of predictor variables (fixed effects) as utilized in the non-spatial model but including random effects for exponential tail-up, exponential tail-down, and Euclidean

autocovariance functions. Comparison of AIC values for the initial non-spatial (AIC = 1649.9) and spatial (AIC = 1608.1) models indicated a spatial mixed model was superior and more appropriate for analyses of abundance.

In the initial spatial model, variance explained by the Euclidean function was 0.4% and the comparison of spatial models with (AIC = 1608.1) and without (AIC = 1596.1) the Euclidean autocovariance function indicated excluding it yielded a better model. Therefore, the Euclidean function was excluded from subsequent spatial models of mussel density. After determining the need to include tail-up and tail-down random effects in a spatial mixed model, the residuals from the initial spatial model were examined. Sixteen records were excluded where logarithmic mussel density was greater than 8. Subsequently the model was iteratively refitted with maximum likelihood estimation and using AIC to guide selection of the final set of predictor variables included in the mixed model.

The best spatial mixed model included two biotic predictors, salmonid biomass and resident trout density (Table 4.2). The random effects in the best spatial mixed model are shown in Table 4.3. Total variance in regional mussel density explained by the spatial mixed model was 52%, with the fixed effects (biotic predictors) explaining 2.4% and the random effects explaining 37.9% and 11.7% for tail-up and tail-down autocovariance functions, respectively (Tables 4.2 and 4.3). The partial sills were 0.85 and 0.26 for the tail-up and tail-down autocovariance functions, respectively, and the respective ranges were 17,480 m and 774 m (Table 4.3).

The data were divided into two subsets representing presence (N = 161) or absence (N = 274) of migratory host fish (Figure 4.6) and separate spatial mixed models of mussel density were analysed. For the subset of records with absence of migratory host fish, the best model of mussel abundance included five fixed effect predictors (Table 4.2, subset 1), three with positive slopes (salmonid biomass, elevation, average annual temperature) and two with negative slopes (resident trout density, slope). Total variance in mussel abundance explained by the mixed model was 50.1%, with the tail-up and tail-down autocovariances accounting for 30.8% and

14.6%, respectively (Table 4.3). The partial sills for tail-up and tail-down functions were 0.74 and 0.35, respectively, and the respective ranges were 12904 m and 158844 m.

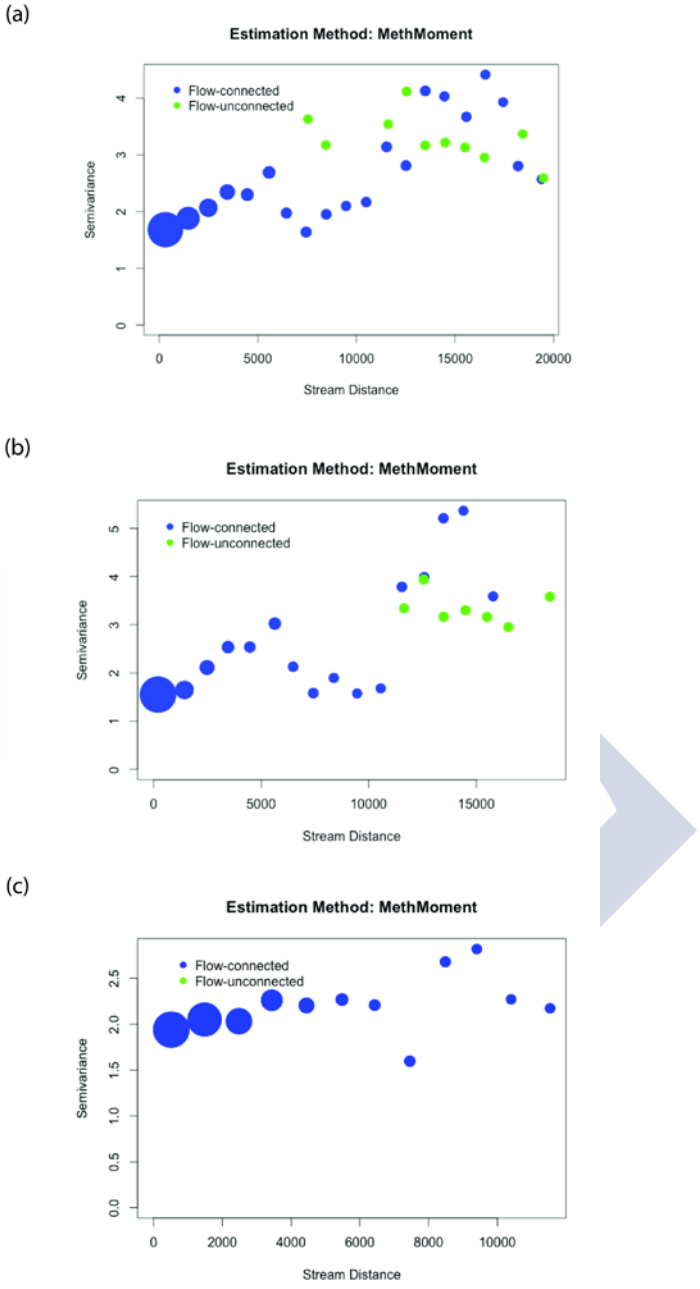


Figure 4.5.- The Torgegrams show the semivariance for mussel density as hydrologic distance (m) between pairs of sites increases; (a) analysis of the full data set (N = 435), (b) the subset of sites with migratory host fish absent (N = 274) and (c) migratory host fish present (N = 161). The size of the circles in each plot is proportional to the number of paired locations used to estimate the semivariance; blue circles indicate flow connected sites and green circles indicate flow unconnected sites. For each Torgegram (a-c), the lower values of semivariance at smaller distances between sites indicate spatial autocorrelation is likely in the model residuals for mussel abundance.

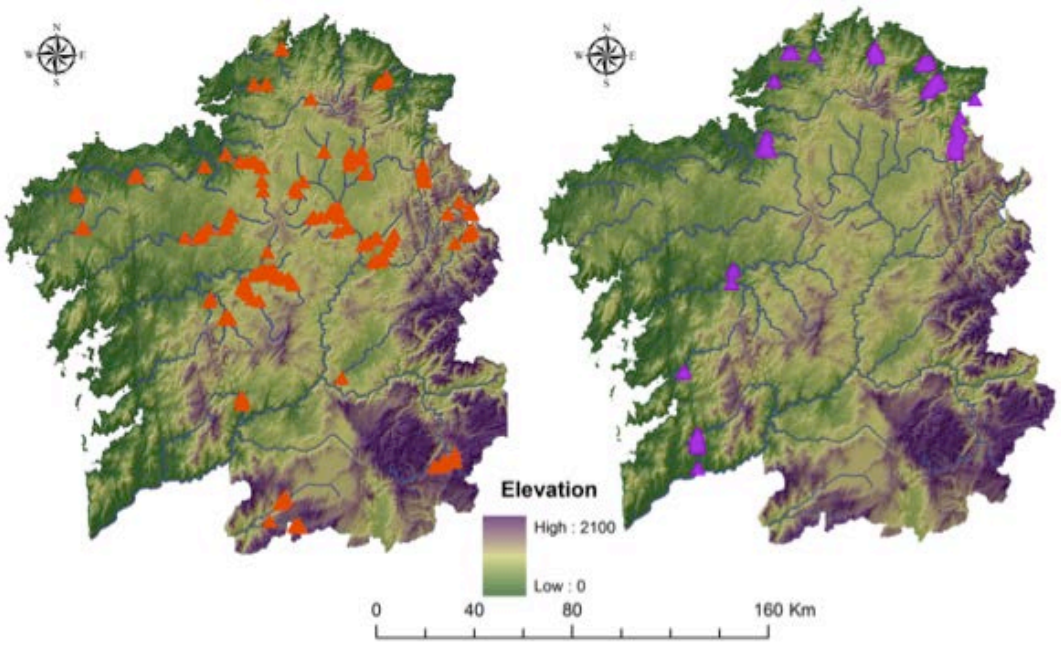


Figure 4.6.- Maps of the *M. margaritifera* density records used for the geostatistical spatial mixed model analyses of subsets of migratory host fish absent (left) and migratory present (right) in the river networks. Orange triangles denote records where migratory hosts fish were absent and purple triangles denote records where migratory host were present.

With the subset of records with presence of migratory host fish, the best model (Table 4.2, subset 2) included predictors for resident trout, detrital rock, forest cover and summer precipitation, which altogether explained 23.4% of the variance in mussel abundance. Random effects in the best model (Table 4.3) explained 23.3% of the variance in mussel abundance for tail-up and 31.4% tail-down. The tail-up function had an estimated partial sill of 1.16 and a range of 114 m, whereas the tail-down function had partial sill of 0.51 and a range of 16,028 m. A total of 78.1% of variance in mussel abundance was explained by the model with presence of migratory host fish.

Data	Parameter	Variance	Estimate	SE[a]	P[b]
Full Dataset[c]	intercept		3.091	0.405	< 0.001
	salmonid biomass		0.195	0.060	0.001
	resident trout density		-0.003	0.002	0.042
	total variance for fixed effects	*2.4 %*			
Subset 1[d]	intercept		-4.080	3.401	0.231
	salmonid biomass		0.112	0.060	0.001
	resident trout density		-0.003	0.066	0.090
	elevation		0.003	0.001	0.028
	slope		-0.021	0.013	0.116
	average annual temperature		0.574	0.237	0.016
	total variance for fixed effects	*4.7 %*			
Subset 2[e]	intercept		7.143	1.102	< 0.001
	detrital rock		-1.072	0.277	< 0.001
	salmonid biomass		0.0235	0.085	0.006
	slope		-0.024	0.014	0.094
	summer precipitation		-0.028	0.007	< 0.001
	total variance for fixed effects	*23.4%*			

Table 4.2.- Estimates for fixed effect predictor variables in spatial linear mixed model analyses of log-transformed mussel abundance for 20 river networks. Results are shown for the full dataset (N= 435) and for two subsets representing absence (N = 274) or presence (N = 161) of migratory host fish. For each analysis, results for fixed effect covariates retained in the best models (AIC-selected) are shown.

[a] SE denotes standard error; [b] probability of obtaining a greater t-value; [c] full dataset; [d] migratory host fish absent; [e] migratory host fish present.

Data	Variance Components[a]	Variance	Partial Sill[b]	Range (m)[c]
Full Dataset	Epanech.tailup	37.9 %	0.8541	17480
	Spherical.taildown	11.7 %	0.2624	774
	Nugget	48.0 %		
Subset 1[d]	Spherical.tailup	30.8 %	0.7379	12904
	Exponential.taildown	14.6 %	0.3502	158844
	Nugget	49.9 %		
Subset 2[e]	Exponential.tailup	23.3 %	1.1580	114
	Epanech.taildown	31.4 %	0.5097	16028
	Nugget	21.9 %		

Table 4.3.- Estimates of random effects in spatial linear mixed model analysis of log-transformed mussel abundance in 20 river networks. Results are shown for the full dataset (N = 435) and for two subsets representing absence (subset 1, N = 274) or presence (subset 2, N = 161) of migratory host fish. The best random effect autocovariance functions (AIC) are shown for each analysis.

[a] variance components in the mixed model are shown for tail-up and tail-down autocovariance functions for river networks; the nugget is an estimate of the variance between sites as distance between them approaches zero; Epanech denotes the Epanechnikov autocovariance function (Garreta *et al.,* 2010); Ver Hoef *et al. (*2006) described exponential and spherical autocovariance functions; [b] the semivariance greater than the nugget; [c] an estimate of the distance between sites in metres at which the semivariance between pairs of sites approaches an asymptotic value; [d] migratory host fish absent, total variance (including fixed effects, Table 4.2) explained by mixed model was 50.1%; [e] migratory host fish present, total variance explained by mixed model was 78.1%.

4.4 Discussion

Discerning the important factors controlling the distribution and abundance of a species is a fundamental question in ecology, conservation, and resource management. In this chapter modelling processes that use advanced techniques to analyse the abundance of the endangered freshwater pearl mussel are presented. The approach yielded significant information about the ecology of this species and the results have considerable relevance for addressing management concerns for habitat protection, reserve allocation, and conservation strategies.

The two techniques applied in this thesis (Geostatistical mixed model and Path anlysis) highlight the importance of including biotic interactions when modelling at regional scale. However, the differences in variance explained by two models (nonspatial and spatial) indicate the necessity of accounting for various sources of spatial autocorrelation when modelling river ecosystems. In this study for mussel abundance, the variance explained for the non-spatial models was 18% for the full dataset, 15% for migratory hosts absent and 28% for migratory hosts present. In contrast for the spatial model, the amount of variance explained was: 52% for the full dataset, 50.1 % for migratory hosts absent and 78% for migratory hosts present.

Moreover, the use of autocovariance functions that account for flow related processes in river ecosystems helped to identify important information regarding biotic interactions. The results show that effects of biotic interactions are discernible at a regional scale in abundance of an endangered freshwater mussel species. In this case study biotic interactions are necessary for mussel populations to persist with successful reproduction, and dispersal by parasitized fish is required to colonize upstream habitat patches and flow-unconnected habitats in a river network.

For the spatial model, host fish biomass and abundance were the only significant predictors of mussel abundance at a regional scale, explaining 2.4% of the variance for the full datset (Table 4.2). Moreover, as Euclidean spatial covariance was discounted for its small model contribution, dispersal of mussels by host fish into flow-unconnected tributaries is suggested by the importance of the tail-down autocovariance (11.7%) (Table 4.3). These results highlight the fact that spatial analysis of biotic interactions can require consideration of models for spatial autocorrelation. Several papers have suggested that spatial autocorrelation is caused by exogenous (e.g. climate, geology) and endogenous processes (e.g. dispersal, reproduction, extinction) (Legendre, 1993; Diniz-Filho *et al.,* 2003; Fortin & Dale, 2005) or by unidentified abiotic variables not included in a model (Keitt *et al.,* 2002). The models presented suggest that biotic interactions with host fish contribute to spatial autocorrelation of mussel abundance in a regional context of multiple river networks.

Rivers are known to exhibit multiple sources of spatial autocorrelation (Peterson & Ver Hoef, 2010). In these data, spatial autocorrelation was apparent between sites separated by up to 17 km (Table 4.3). Where migratory ecotypes of host fish co-occurred with mussels, mussel abundance was more predictable (78.1% variance explained) and tail-down spatial covariance was larger (31.4%) (Table 4.3). Thus, the spatial mixed model approach helped to estimate different process-based contributions to spatial autocorrelation and it provided an answer to the central question on the spatial effect of biotic interactions on species abundance.

This study illustrates a way to consider biotic interactions in modelling abundance and for refining conservation plans for parasite-host systems. As freshwater mussel species are imperilled worldwide, this study highlights the importance of macroecological studies to reduce extinction risk for freshwater mussels.

Modelling Process Considerations

Stratification of the data, and conducting analyses between and within domains revealed substantially heterogeneous habitat conditions in different rivers. Spatial heterogeneity that inflates residual variance can be associated with different domains where topography, climate history and geologic setting govern disturbances that influence riverine community structure and dynamics (Montgomery, 1999). Alternatively, anthropogenic perturbations in different rivers and stream segments within rivers can be a source of spatial heterogeneity. I acknowledge that additional factors not included in the analyses (e.g. human impacts, landscape modifications) affect abundance of an endangered species such as *M. margaritifera* at different temporal and spatial scales within rivers. Thus, various stratifications into domains differed in the level of essential information they provided.

The model procedures described in this chapter helped to identify sources of spatial heterogeneity when trying to discern spatial patterns, although there were some limitations in its application. First, stratifying the data into domains requires

sufficient records so that individual domains are large enough for analyses to be conducted. In this study, in two instances of domains with 80 or fewer records where path analysis could not be conducted (see Annex IV). Second, some knowledge of the landscape and species under study is necessary to choose relevant environmental predictors and to decide ways to stratify the data into domains. Moreover, the application of spatial analytical techniques for stream networks requires advanced GIS and statistical skills along with knowledge of freshwater ecology (Peterson & Ver Hoef, 2014). Moreover, some spatial models did not have a solution in R, as in the subset with migratory host fish absent where singular covariance matrices were observed for some models. Further developments in spatial network analysis are needed to accommodate asymmetry in river connectivity caused by dams and other barriers that limit upstream movement of organisms but that can allow downstream movements. For example, there is no way presently to split a stream network at dams for analysis with the SSN package. As a result, there is no direct way to handle network fragmentation. Data availability for parasite-host or similar biotic interactions is a potential limitation on the spatial extent to which the analyses can be applied. Moreover, in this specific study case, as *M. margaritifera* has a longer life span than its hosts, its current abundance may have a direct link with the historical abundance of its host fish. Options to overcome this limitation might include, for example, developing a predictive model for the host, and using subsequent model predictions in modelling abundance of the parasite.

Non-Spatial Model

The path analysis was used to analyse abundance of the freshwater pearl mussel for two reasons. First, it was important to know how much variance could be explained with broad scale factors that are often used in MaxEnt models of species distribution. Second, path analysis provided an informative way to view direct and indirect effects of individual predictor variables, as well as the relative importance of the different categories of predictors.

For regional mussel abundance, salmonid biomass and density of resident trout were the only important biotic predictors. In contrast, the relative importance of fish hosts varied with scale in the analyses of abundance. It identifies a discontinuity in biotic interactions affecting spatial patterns of mussel abundance at a broad scale. Where migratory hosts were absent, production and density of resident trout had positive direct effects on mussel abundance but their effects were much smaller than temperature and elevation, and overall very little variance in mussel abundance was explained (Figure 4.3). With presence of migratory hosts (Figure 4.4), the variables for fish hosts had larger direct effects and were nearly equal in importance to landform. Results indicated that analysis of species abundance can fail to identify important broad scale information when the spatial heterogeneity and life traits of biotic interactions between a parasite and its host are not considered. I was primarily interested in quantifying spatial patterns in mussel abundance attributable to broad scale abiotic and biotic factors, and I acknowledge that more factors may affect mussel abundance at a local scale (eg. physicochemical characteristics of riverbed substratum (Geist & Auerswald, 2007; Quinlan *et al.*, 2014).

For path analysis applied to the full data set the low amount of variance explained (18.2%) indicated some effects of climate and landform on mussel abundance with little contribution from host fish and geology. This result seemed inconsistent with other reports on the ecological prevalence of *M. margaritifera* in metamorphic and granitic geologic areas (Bauer, 1986; Geist & Auerswald, 2007) and the species dependence on host fish to complete its life cycle (Hastie & Cosgrove, 2001; Arvidsson *et al.*, 2012).

Subsequent analyses by presence or absence of migratory salmonids showed that fragmentation of rivers by dams is a source of reduced mussel abundance, with higher mussel abundance where migratory fish hosts occurred. Dams have excluded the Atlantic salmon and migratory trout from upstream areas of many rivers in the northwest portion of the Iberian Peninsula (Hervella & Caballero, 1999, 2002; Caballero *et al.,* 2006, Garcia de Leaniz, 2008). These results suggest that extinction of

migratory fish hosts upstream of dams may have created a coextinction debt for many isolated populations of *M. margaritifera* in the study area. Elsewhere, extinction of mussels from several river systems and upstream of dams has been linked to loss of appropriate host fishes (Kat & Davis, 1984; Watters, 1996).

Spatial Model

The spatial models used in this study for analysis of abundance account for the processes that occur in river ecosystems (Peterson & Ver Hoef, 2010). There are three essential features of any river that can lead to spatial autocorrelation between sites within river networks. First, downstream flow of water is an ecosystem process that transports dissolved and suspended materials including organisms derived from the river itself or from the surrounding landscape (Ward *et al.,* 2002; Wiens, 2002); this is a tail-up source of autocovariance in a stream network (Cressie *et al.,* 2006, Ver Hoef *et al.,* 2006). Second, upstream movement of organisms against the river flow is an ecological process that can cause autocorrelation within a river network, which represents a tail-down autocovariance (Ver Hoef *et al.,* 2006; Ver Hoef & Peterson, 2010). Tail-down functions are applicable to upstream movement of organisms against the flow as fish do (Cressie & O'Donnell, 2010). The third source of spatial autocorrelation in river networks is Euclidean rather than actual stream distance separating sites and it is the usual autocorrelation considered in terrestrial spatial analyses; sites closer together may share similar levels of disturbance from human activities in the riverscape and/or they may share similar abiotic regimes with influences from climate, land form and geology for instance.

Because adult mussels are largely sedentary organisms with limited ability to move upstream against the flow, it was of special interest to evaluate if there is a tail-down component of spatial autocorrelation that might link upstream dispersal of the mussel by its host fish during the mussel's parasitic glochidial stage. In these results the tail-down component was an important part of spatial autocorrelation of mussel abundance in river networks (Table 4.3). Thus, it seems that the tail-down component of spatial autocorrelation is likely to be a result of biotic interactions, earlier in time,

where upstream mussel dispersal by parasitized host fish occurred into flow-unconnected tributaries. As it was described for other mussel's species of the family Margaritiferidae, population size of mussels was dependent on the upstream reproductive subpopulations and on upstream tributaries, due to its asymmetrical dispersal (Terui *et al.*, 2014). These results show clearly that the tail-down component representing biotic interactions is important.

In contrast, the tail-up component of spatial covariance is larger (Table 4.3), and it can include more varied modes of downstream mussel dispersal, such as downstream displacement of mussels by high river flows (Hastie *et. al.*, 2001) downstream dispersal of parasitic glochidia and downstream movements of parasitized host fish that disperse juvenile mussels to downstream habitats. Thus, the tail-up component may also include a contribution from biotic interactions. Tail-up spatial autocorrelation in mussel abundance may also indicate general zones within a river network where dissolved and suspended organic carbon resources are sufficient to support filter-feeding invertebrates (Vannote *et al.,* 1980). With the substantial variance components associated with the tail-up and tail-down functions, it seems likely that spatial autocorrelation across the study region of 20 riverscapes is caused in part by biotic interactions.

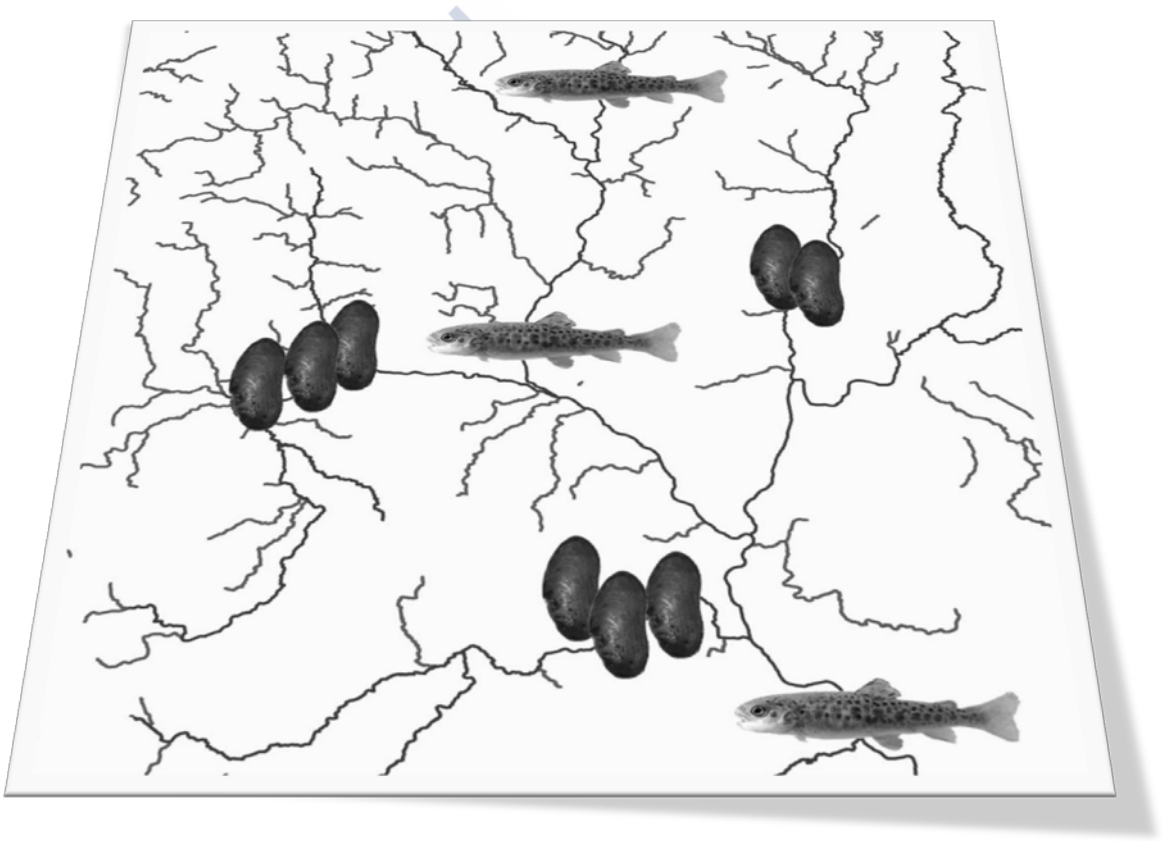
Analysis of subsets of data approximated the influence of dams on availability of migratory host fish. Mussel abundance was more predictable (Tables 4.2 and 4.3) where migratory fish were present (Figure 4.6), suggesting that rivers that support migratory host fish are prime areas for finer scale study of factors regulating local scale mussel abundance. In the second chapter evidence of recent reproduction of *M. margaritifera* was reported in 11 rivers in the study area, six of 14 rivers (43%) with presence of migratory host fish and five of 38 rivers (13%) with resident trout as the only available host. This is a significant difference (Fisher's exact test, P= 0.0105) in mussel reproductive success associated with presence of migratory host fish.

Dispersal biology of host fish appears to be important for mussel dispersal (Schwalb *et al.*, 2011). The results from this study suggest that migratory host fish are

more important than resident host fish for several reasons. First, *M. margaritifera* generally has higher infection rates in age 0 and older juvenile hosts (Hastie & Cosgrove, 2001). Second, the larger body-sized migratory salmonids may facilitate successful mussel reproduction because the larger sized migratory salmonids produce substantially more juvenile hosts for the mussel (Pope *et al.,* 1961; Beacham & Murray, 1993; Elliott, 1995; Fleming, 1996; Dickerson *et al.,* 2002; Milner *et al.,* 2003; Cowley, 2008). Third, migratory host fish make more extensive movements in the river than do resident trout (Milner *et al.,* 2003). Hence, movement of parasitized migratory hosts may be a more effective mechanism for long distance mussel dispersal than is provided solely by resident trout. This fact may account for part of the regional scale importance of biotic interactions in these analyses. In general, distribution and movement patterns of host fish have been suggested to influence the distributions of several freshwater mussel species (Watters, 1996; Vaughn, 1997; Haag & Warren, 1998; Vaughn & Taylor, 2000); the analyses presented in this study add a new perspective to these views.

How much variation in species abundance might be caused by biotic interactions? Direct, regional scale effects of the biotic predictors accounted for about ~3% of mussel abundance. Much of the tail-down autocovariance may represent biotic interactions (~10%) because Euclidean autocovariance was unimportant in the model. Finally, tail-up autocovariance (~30%) can also include downstream dispersal by host fish (Tables 4.2 and 4.3). Thus, biotic interactions could account for one-fourth of the variance in parasite abundance and as much as half of the spatial autocorrelation.

5. SPATIAL MODELS TO ADDRESS CONSERVATION OF INTERACTING SPECIES IN RIVER ECOYSTEMS



”Os que dormen nun colchón vólvense da mesma condición”

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DE SANTIAGO
DE COMPOSTELA

5. SPATIAL MODELS TO ADDRESS CONSERVATION OF INTERACTING SPECIES IN RIVER ECOSYSTEMS

5.1 Introduction

It is widely recognized that conservation of biodiversity is facilitated by maintaining population densities and distributions of strongly interactive species (Soulé *et al.*, 2005). In this framework, there is growing concern that parasite and mutualistic species represent a coextinction crisis (Stork & Lyal, 1993; Koh *et al.,* 2004; Dunn *et al.,* 2009). Coextinction in a host-parasite system is defined as the loss of a species (the affiliate) upon the loss of another species (the host) (Koh *et al.,* 2004). Models predict that extinction of a host species could lead to extinction of one or more parasite species, so that parasite extinctions could equal or exceed the number of host extinctions. Thus coextinction should be a primary consideration for preventing cascading biodiversity loss associated with interactions between species (Dunn *et al.,* 2009).

River environments are dendritic ecological networks (Peterson *et al.*, 2013) where flow has an important role on controlling biotic interactions between species. Moreover, their dendritic spatial structure may influence the patterns in which species interactions occur. Thus, models that account for spatial characteristics of river ecosystems are necessary to address conservation of interacting groups such as the parasite-host systems between the freshwater mussels and their hosts. However, no broad scale study has been carried out to understand the role of parasite-host interactions influencing abundance patterns of freshwater mussels. Here, a case study to address conservation in the framework of interacting species by using *M. margaritifera* is presented. This work describes a two-step approach by using (1) a geostatistical interpolation technique (kriging) to predict mussel abundance and (2) analyses of demographic structure of its populations to define conservation strategies to maintain mussels and their host fishes.

5.2 Materials and Methods

Abundance Prediction

Abundance prediction was made using kriging (Krige, 1966). Kriging is a geostatistical interpolation technique that is based in the previous knowledge of the spatial dependence in the data (Fortin & Dale, 2005). It is a weighted moving average technique that uses the geostatistical spatial parameters of range, nugget and sill estimated previously by the spatial models in Chapter 4. In the framework of geostatistics (Matheron, 1963) the models described estimate the maximal variance (the sill) between a pair of uncorrelated sites, partition it into variance (partial sill) attributable to each source of spatial covariance, and the distance at which spatial autocorrelation occurs (geostatistical range) for each spatial component (see Table 4.2 for the estimated values).

In this chapter, abundance prediction was made using universal kriging with the SSN package in R (R Development Core Team, 2010). Model predictions were carried out at 1 Km points and 435 abundance sites (Figure 5.1) by using the covariance parameters estimated in model developed in Chapter 4 for the full dataset. Mussel abundance predictions were displayed by using Arcmap 10.2 for the 20 river networks.

Population Structure

Different age profiles were developed for 13 rivers by using length–age keys. The procedures described below were carried out in the rivers where higher abundance of the species occurred (see Chapter 2, Figures 2.7 and 2.8). A minimum of 50 randomly selected mussels per site was measured in at least 5 locations per river using a waterproof digital caliper (Figure 5.2). The empty shells (encountered during the Phase I sampling described in Chapter 2) were collected and used to determine the length of the specimens at different ages. These data were used to construct a length–age key for each river following the methodology described in Outeiro *et al.* (2008).

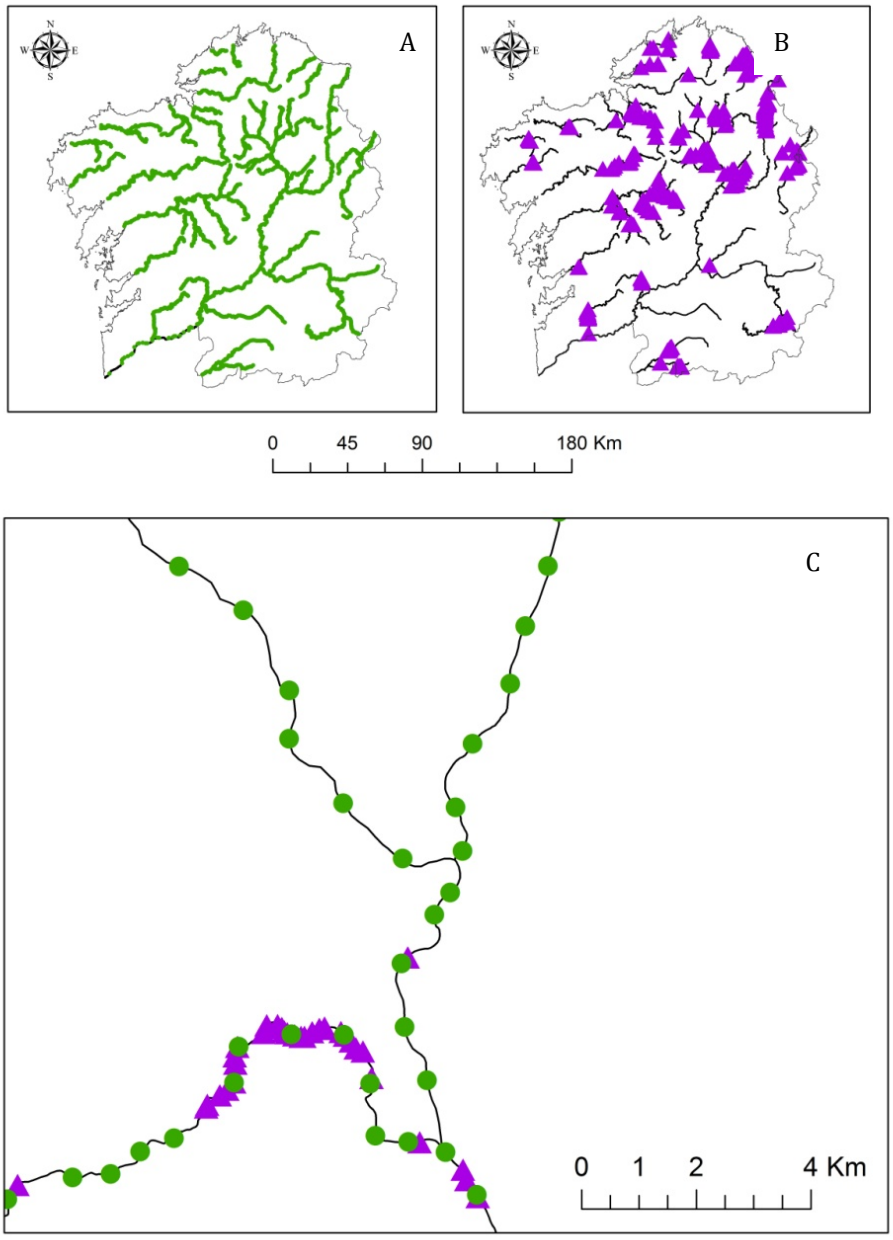


Figure 5.1.- Distribution of abundance sites and prediction points in the river networks of the study area. (A) Green areas represent sites were density was recorded. (B) Purple triangles represent prediction points at 1 km. (C) Detail of prediction points (green dots) and density sites (purple triangles).

Figure 5.2.- Pictures of length measurements of individuals of different ages in the field.

In order to obtain the age-length relationships for each population, the periostracum of the shell was removed with a KOH solution and annual growth rings were counted (Ekman, 1905 cited in Ziuganov *et al*., 1994) (Figures 5.3 A, B and C). To estimate the early rings when the adult mussels' umbo is erased, data measurements of alive juveniles were used to determine the age of the first discernible ring in empty old shells (Bauer, 1992; Ziuganov *et al.,* 1994). A minimum of 30 shells per river were photographed and measured by using ImageJ software (Abràmoff *et al.*, 2004) to obtain data on length-age relationships for each population. Moreover, several shells from different rivers were examined by using the technique called Mutvei´s solution (Schöne *et al*., 2005) (Figures 5.3 E and F) to determine the age of growth rings of some specimens. A length–age key for each river was developed and age–frequency distributions were obtained to describe population structure. For population status assessment the following criteria were used: sites with presence of mussel recruitment in the last 5 years and sites where 20% of individuals are between 5 and 10 years. These criteria were based on growth curves developed by San Miguel *et al.* (2004) for Galicia populations, and they are consistent with the criteria proposed by Moorkens *et al.* (2007) to evaluate recruitment.

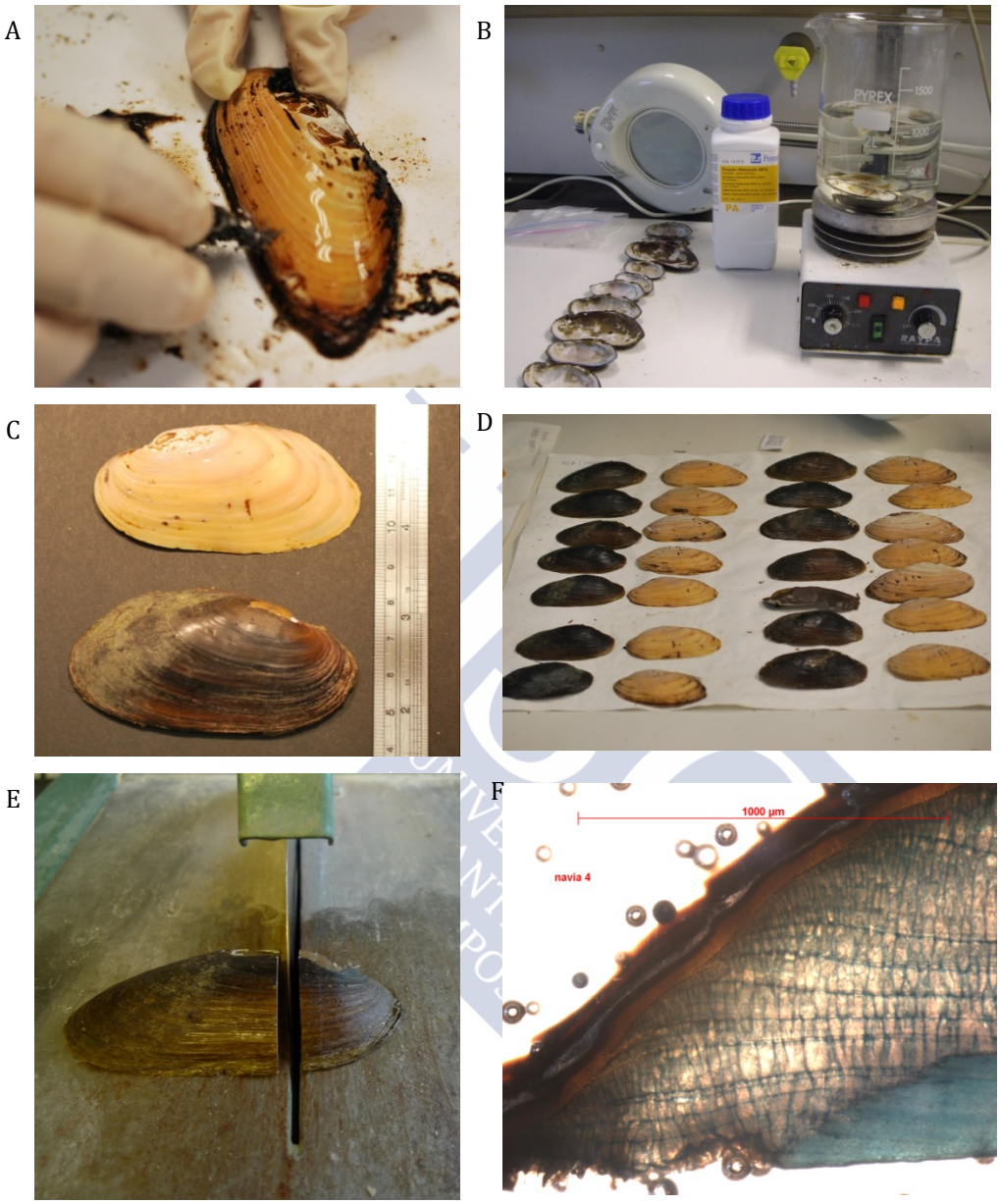


Figure 5.3.- Techniques for determing the age-lengh relationships for *M. margaritifera* populations (A, B, and C), (D) process of periostracum removal, (E) cutting shells, and (F) detail of growth rings in Mutvei´s solution. Phtos by Sabela Lois.

Conservation Zonation

Conservation strategies were identified for several areas by combining the abundance predictions and the population structure assessments for 65 sites of higher abundance within 13 rivers (Figure 6.1). The criteria used for defining conservation zones were: (1) presence or absence of sites in which host-parasite interactions occurred successfully currently, leading to the existence 20% of individuals between 5 and 10 years and (2) presence or absence of migratory host fish.

5.3 Results

Abundance Predictions

Spatial model predictions ranged from 11.2 to 305.6 mussels per 40x40 m pixel size for the 20 drainage basins (Figure 5.4). Standard errors varied from 0.002 to 0.035. The predictions with lowest standard errors were in sites where the higher mussel abundances were predicted (Figure 5.4). The abundance prediction values were organized into categories in order to display abundance patterns on a map. Abundance prediction values varied in the areas where migratory hosts fish were present (Figure 5.5). Categories were constructed based on abundance data distribution and they ranged in number of individuals per 40x40 m pixel size:

<50	Low
50 to 124	Medium
125 to 300	High
>300	Very high

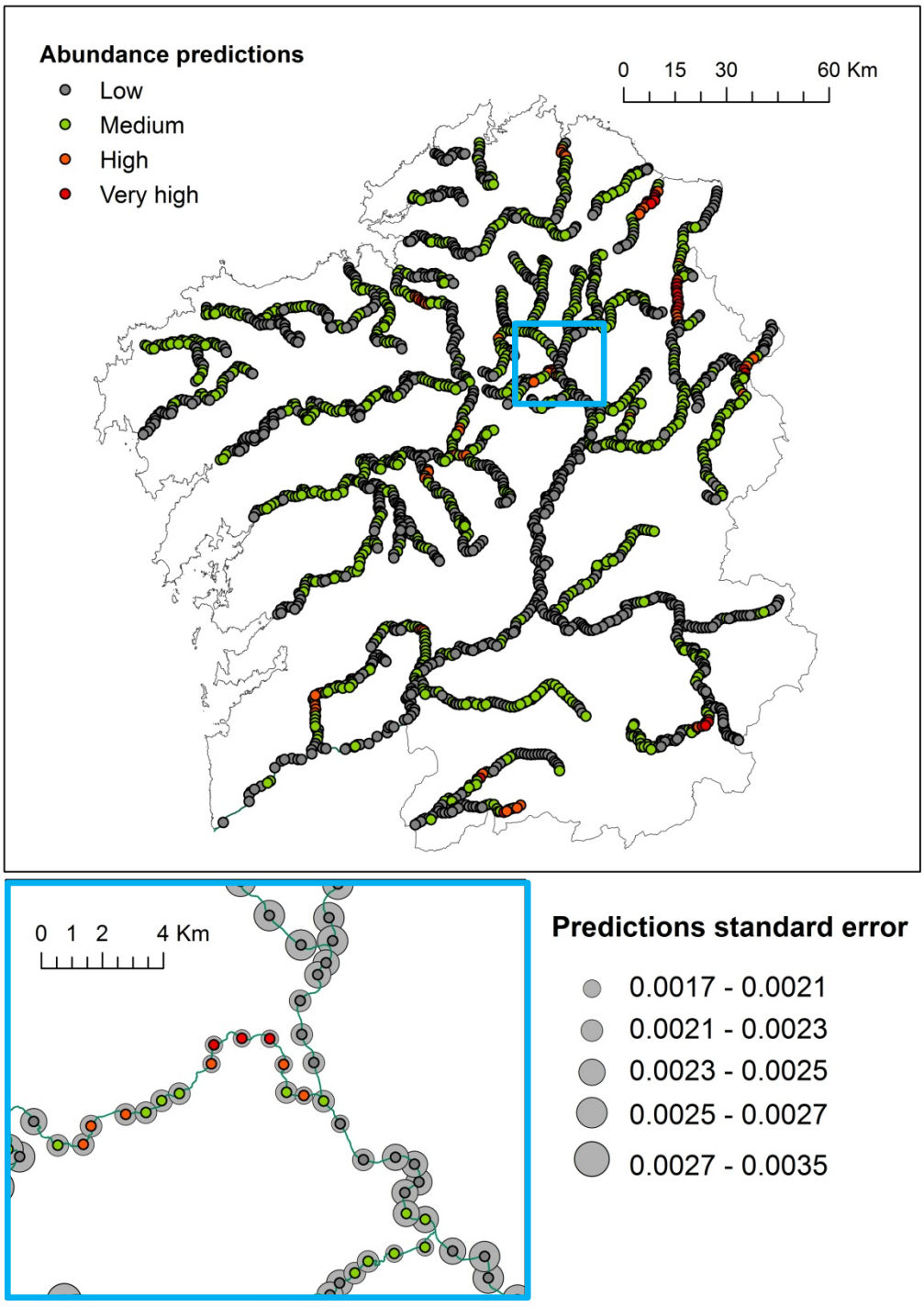


Figure 5.4.- Mussel density predictions (individuals/40x40 m) organized in categories <50 (Low), 50 to 124 (Medium), 125 to 300 (High) and >300 (Very high). The detailed map shows in grey the standard errors for each prediction.

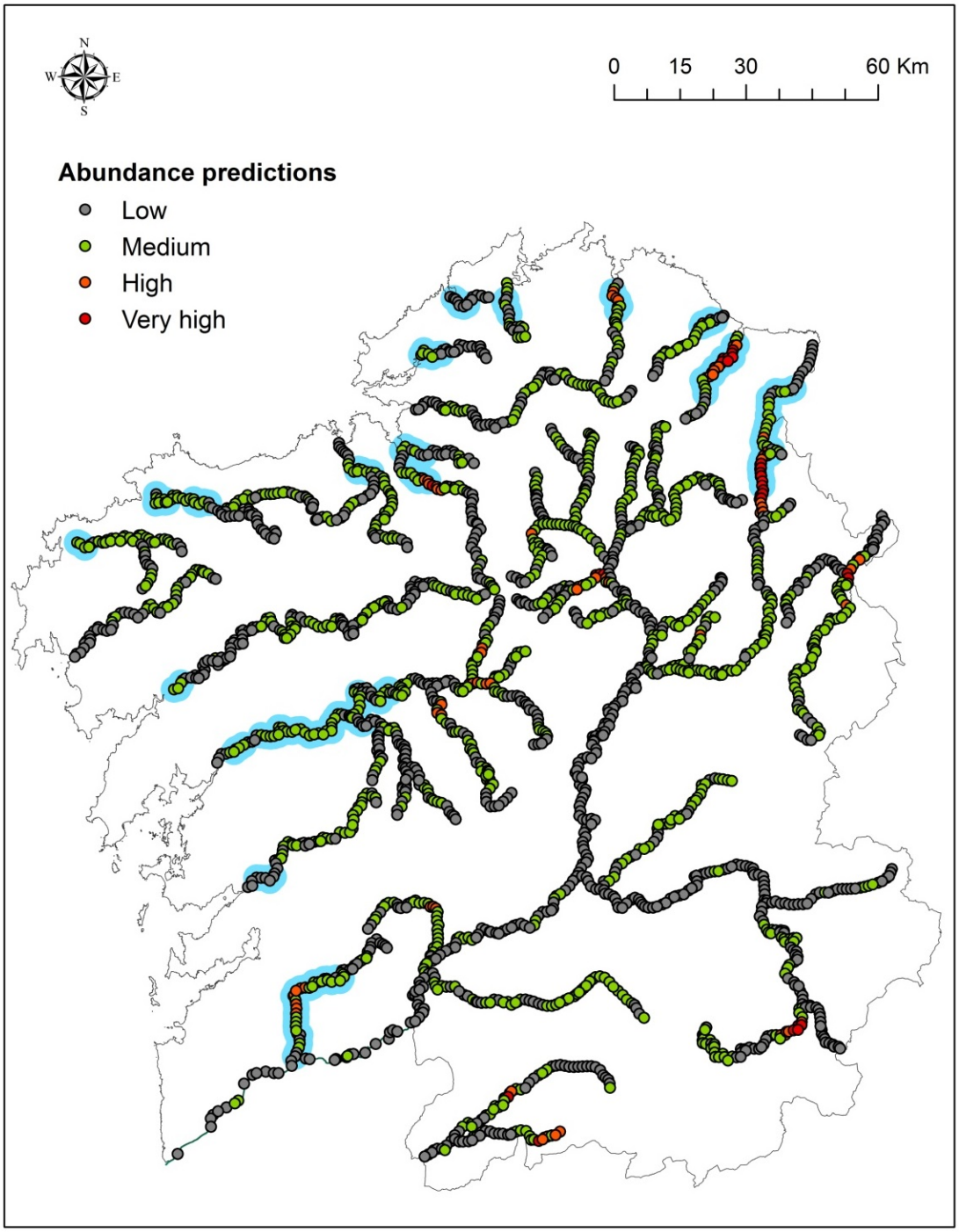


Figure 5.5.- Abundance predictions organized in categories as in Figure 5.4. The areas with blue background represent areas with presence of migratory fish.

Population Structure

Age profiles of *M. margaritifera* showed different shapes depending on the river network analysed. Several sites within rivers showed where recruitment has occurred in the last 5 years (Limia, Tea) (Figures 5.6 and 5.7). Furthermore, Tea, Limia, Camba and Eo and had at least one site in which 20% of the population was comprised of individuals between 5 and 10 years of age. In contrast the remainder of the river sites showed a population structure dominated by old individuals. Moreover as a general trend, age-classes between 15 and 25 years were the most abundant individuals in the majority of the sites within rivers. The age profiles for each site ere shown in Figure 5.7.

According to San Miguel *et al.* (2004), the populations of *M. margaritifera* in Galicia show the shortest lifespan of the distribution range of the species. In the study area, certain populations such as Tea and Limia, have their oldest individuals about 25 years of age. In contrast, their length profiles are similar to the other populations (see the length frequency profiles in Annex V Figure V.1), suggesting that they may have a higher growth rate and a shorter lifespan than the populations described by San Miguel *et al.* (2004).

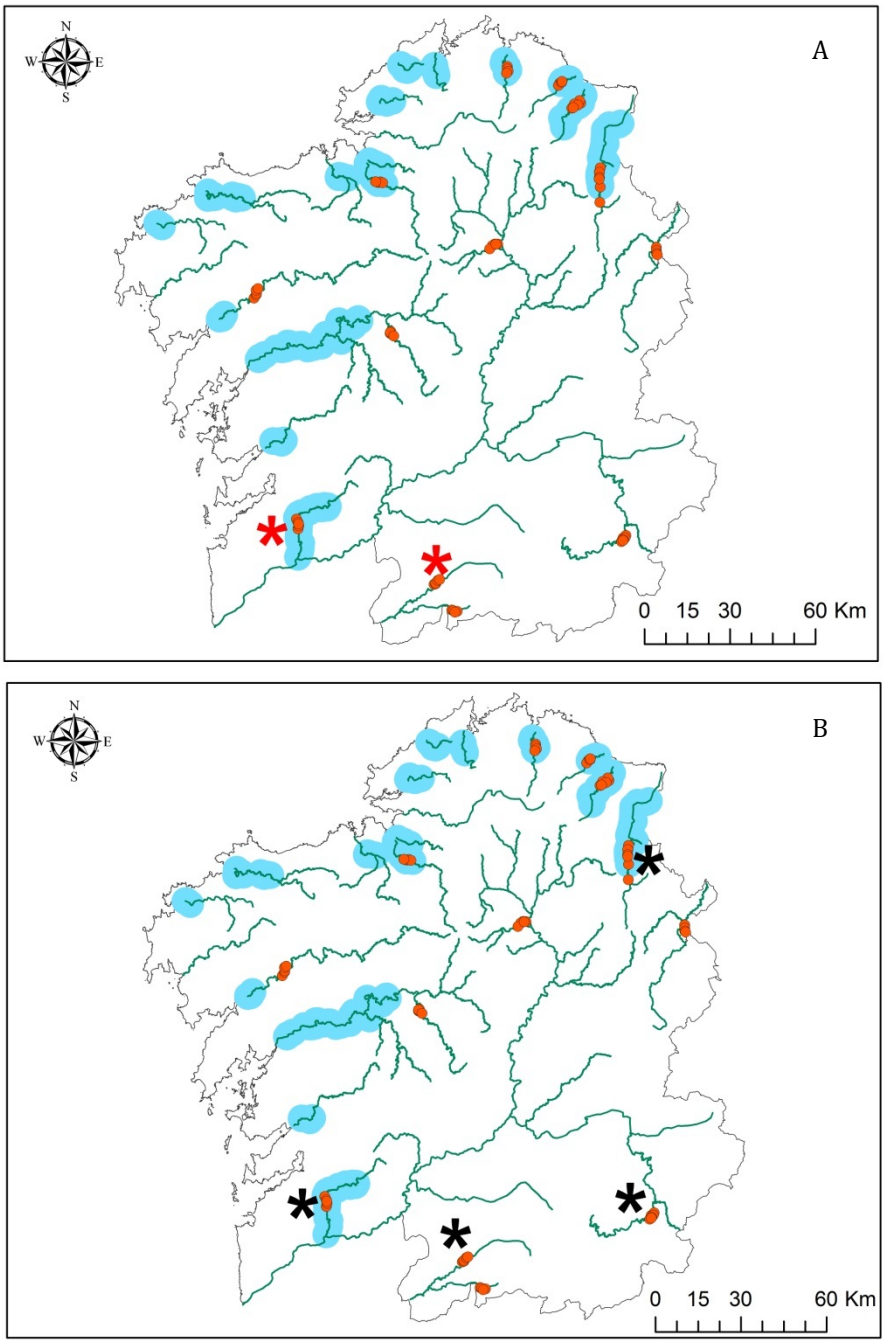
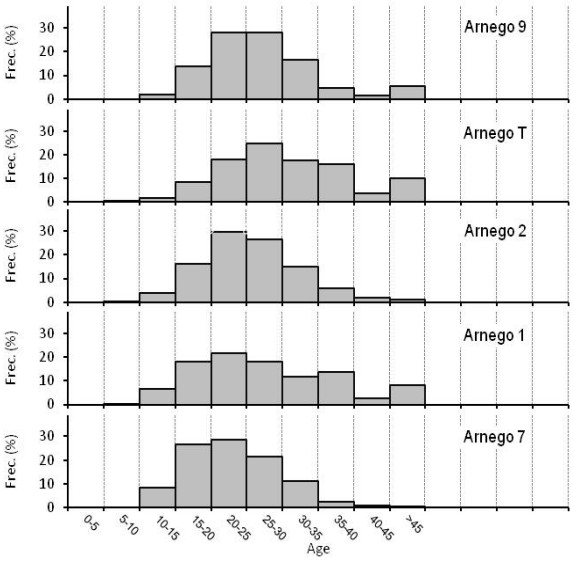
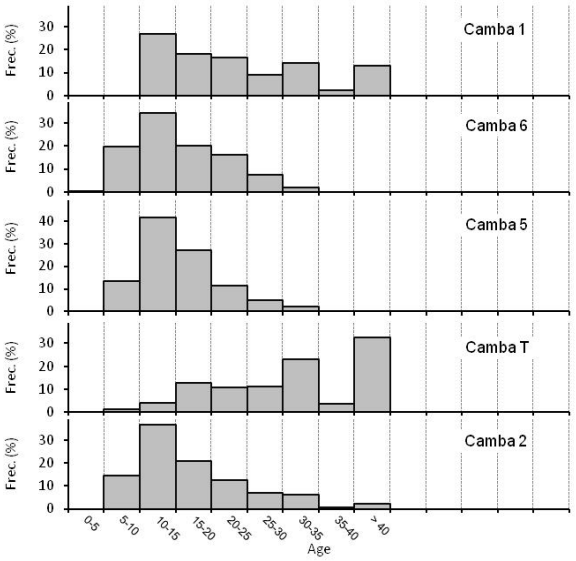
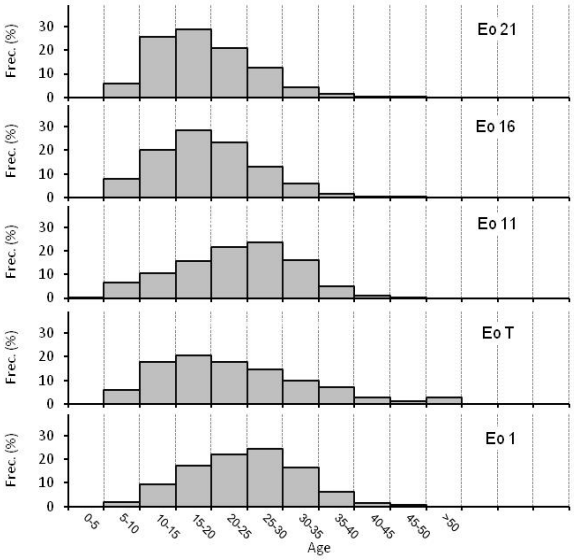
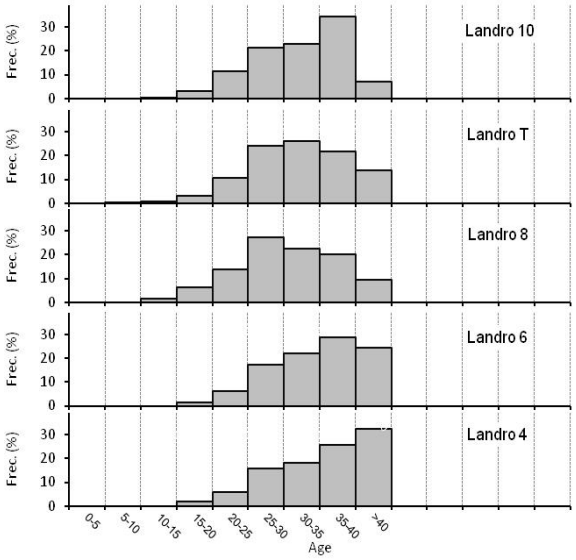


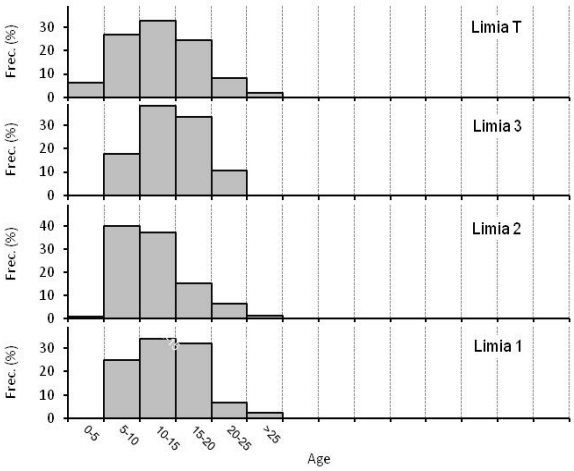
Figure 5.6.- Sites of high abundance where population structure assessment was conducted (A) Red asterisks represent sites where mussels less than 5 years were found (B) Black asterisks denote rivers that had sites with at least 20% of individuals between 5-10 years. Blue areas denote presence of migratory hosts and red dots represent mussel density records.

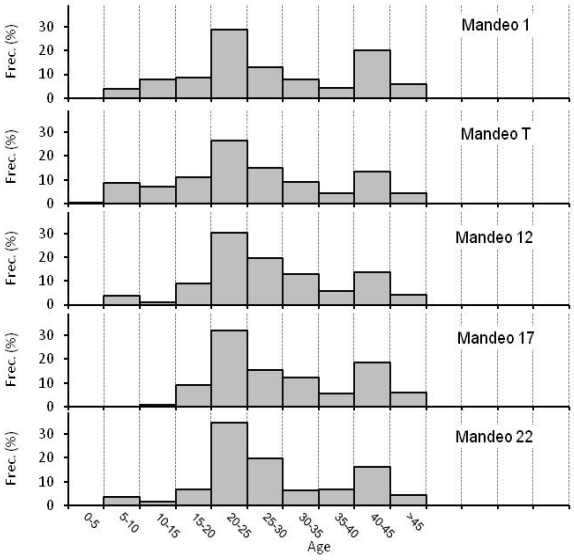

Arnego 9
Arnego T
Arnego 2
Arnego 1
Arnego 7
Frec. (%)
0 10 20 30
0-5 5-10 10-15 15-20 20-25 25-30 30-35 35-40 40-45 >45
Age

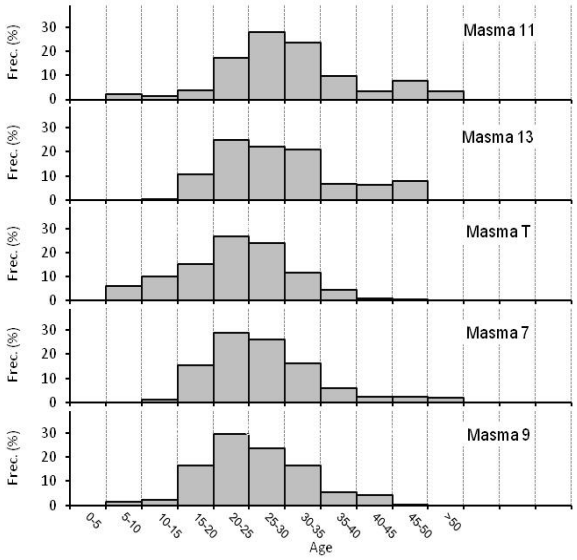

Camba 1
Camba 6
Camba 5
Camba T
Camba 2
Frec. (%)
0 10 20 30 40
0-5 5-10 10-15 15-20 20-25 25-30 30-35 35-40 > 40
Age

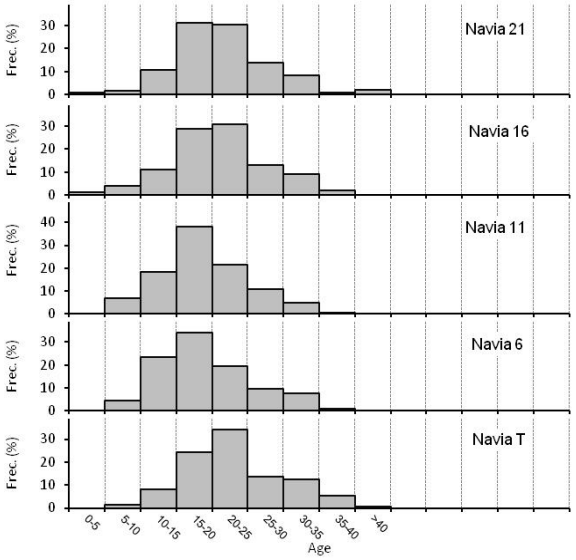

Eo 21
Eo 16
Eo 11
Eo T
Eo 1
Frec. (%)
0 10 20 30
0-5 5-10 10-15 15-20 20-25 25-30 30-35 35-40 40-45 45-50 >50
Age

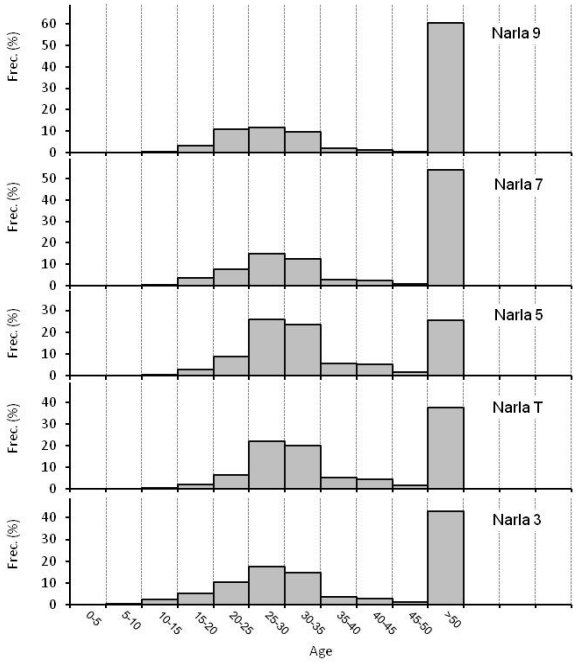

Landro 10
Landro T
Landro 8
Landro 6
Landro 4
Frec. (%)
0 10 20 30
0-5 5-10 10-15 15-20 20-25 25-30 30-35 35-40 >40
Age

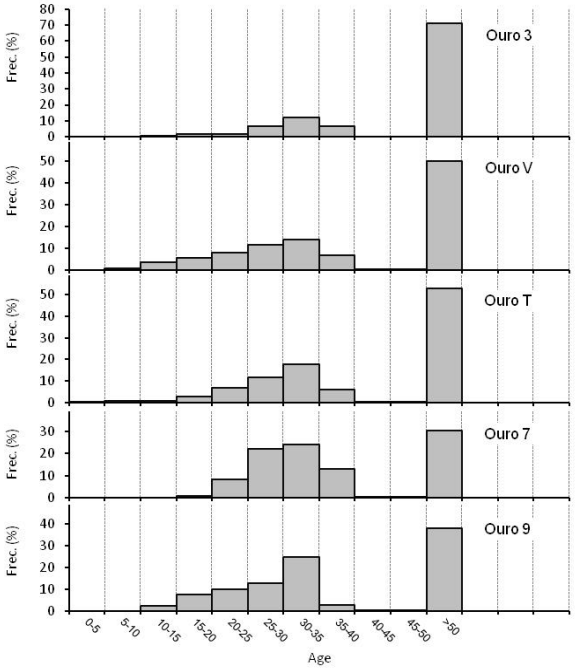

Limia T
Limia 3
Limia 2
Limia 1
Frec. (%)
0 10 20 30 40
0-5 5-10 10-15 15-20 20-25 >25
Age

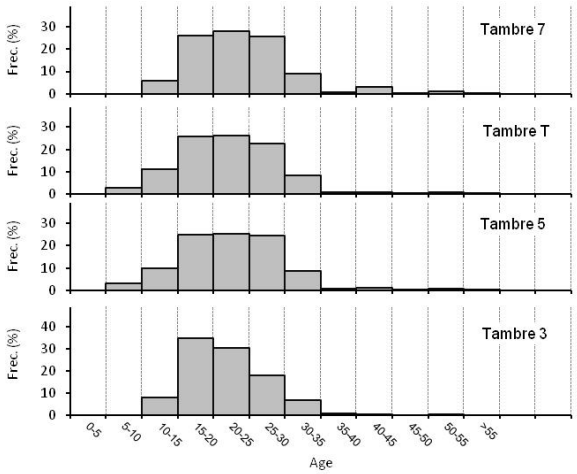

Mandeo 1
Mandeo T
Mandeo 12
Mandeo 17
Mandeo 22
Frec. (%)
0 10 20 30
0-5 5-10 10-15 15-20 20-25 25-30 30-35 35-40 40-45 >45
Age

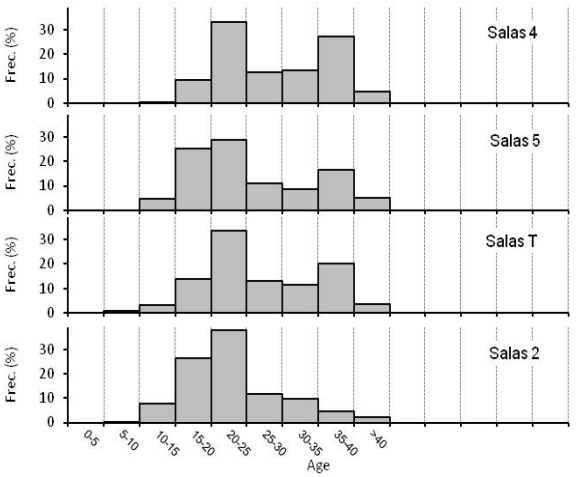

Masma 11
Masma 13
Masma T
Masma 7
Masma 9
Frec. (%)
Age
0-5
5-10
10-15
15-20
20-25
25-30
30-35
35-40
40-45
45-50
>50


Navia 21
Navia 16
Navia 11
Navia 6
Navia T
Frec. (%)
Age
0-5
5-10
10-15
15-20
20-25
25-30
30-35
35-40
>40


Narla 9
Narla 7
Narla 5
Narla T
Narla 3
Frec. (%)
Age
0-5
5-10
10-15
15-20
20-25
25-30
30-35
35-40
40-45
45-50
>50


Ouro 3
Ouro V
Ouro T
Ouro 7
Ouro 9
Frec. (%)
Age
0-5
5-10
10-15
15-20
20-25
25-30
30-35
35-40
40-45
45-50
>50


Tambre 7
Tambre T
Tambre 5
Tambre 3
Frec. (%)
Age
0-5
5-10
10-15
15-20
20-25
25-30
30-35
35-40
40-45
45-50
50-55
>55


Salas 4
Salas 5
Salas T
Salas 2
Frec. (%)
Age
0-5
5-10
10-15
15-20
20-25
25-30
30-35
35-40
>40

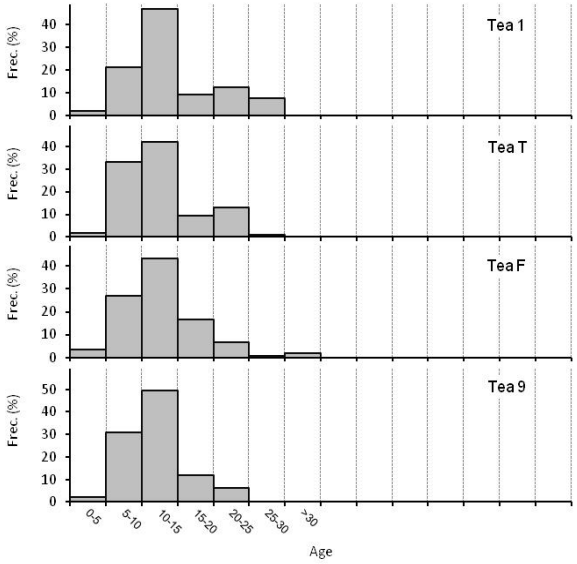


Figure 5.7.- Age frequency distributions of *M. margaritifera* for different sites within 13 rivers studied. Each number denotes a different sampling site and sites named with a T at the end of river name represents the 13 sites where microhabitat measurements were conducted (see Chapter 6).

Conservation Zonation

Combining age profiles, presence or absence of migratory host fish and abundance predictions (Figures 5.6, 5.7 and 5.8) from the analyses in this thesis yielded four categories of conservation zones, which were defined based on the presence of migratory hosts fish and the population profiles. In this way, four conservation strategies (zones) were defined for the 13 populations of *M. margaritifera* and its fish hosts (Figure 5.9).

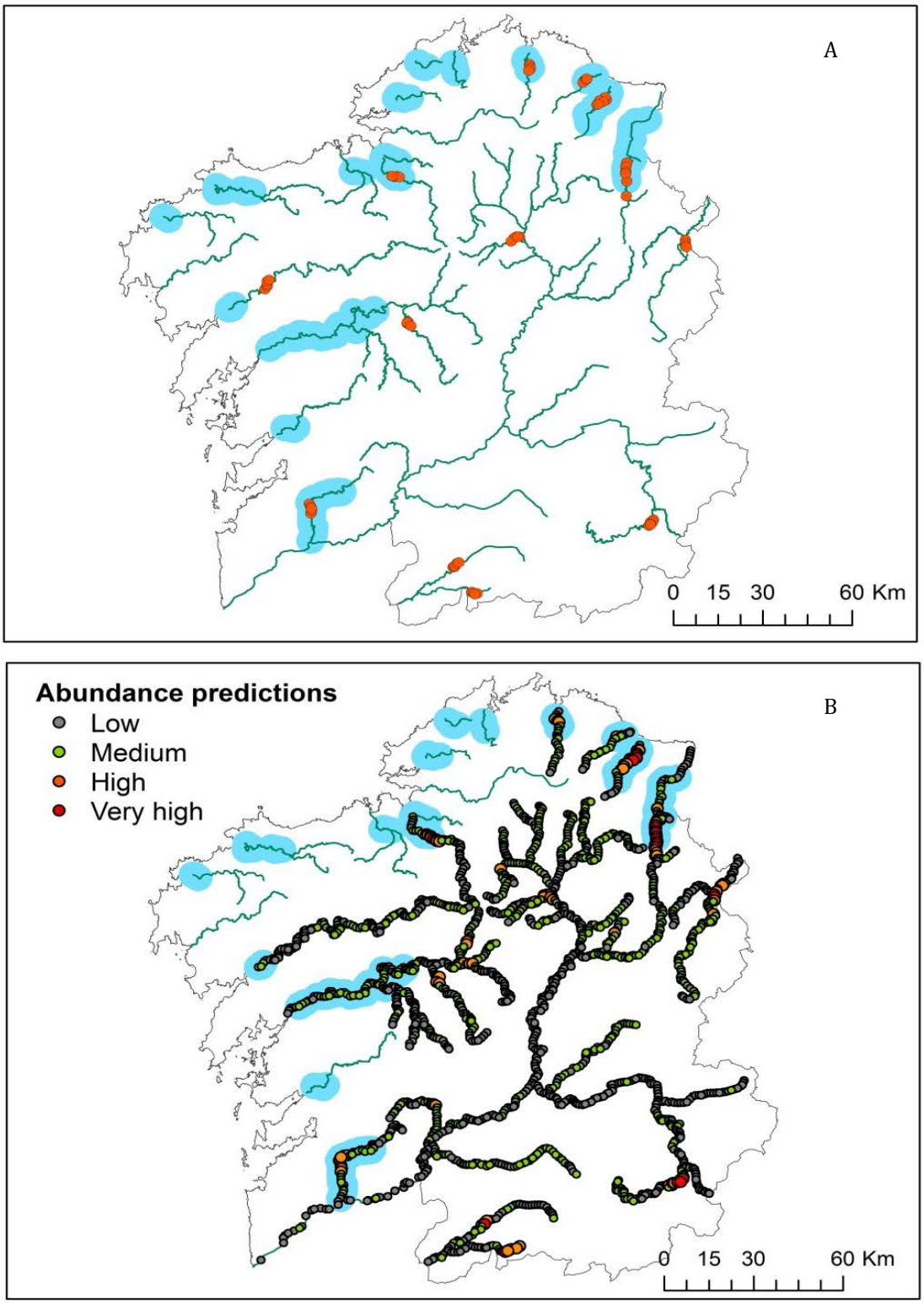


Figure 5.8.- River networks where several age profiles were obtained and mussel abundance was predicted by a spatial mixed model. (A) Sites of high abundance where population structure assessment was conducted and (B) predictions for networks in which age profiles were determined. Blue areas denote presence of migratory hosts.

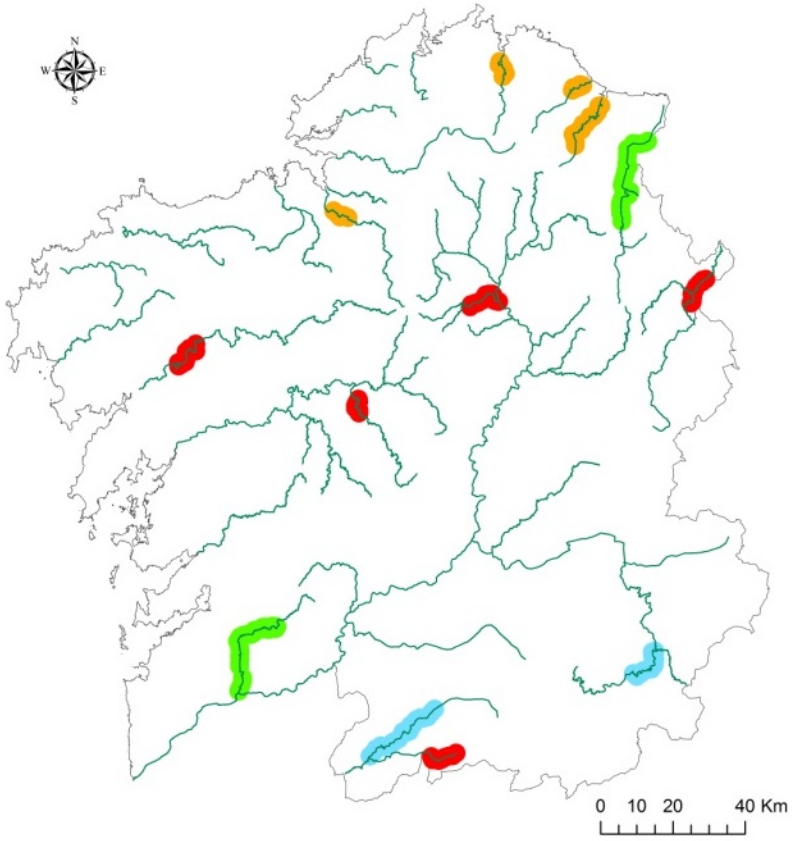


Figure 5.9.- Location of conservation zones within the study area are shown in the map, the table shows the color codes for different areas and the conservation criteria and strategies.

Conservation criteria				***Conservation strategies***	
Presence of migratory host fish	*Presence of recruitment*	Rivers	Basins	*Objetives*	Color code
1	1	Eo, Tea	Eo, Miño	Protection and Monitoring	
0	1	Camba, Limia	Miño, Limia	Protection and Monitoring especially on host fish populations	
1	0	Landro, Ouro, Mandeo, Masma	Landro, Ouro, Mandeo, Masma	Habitat assessment	
0	0	Navia, Tambre, Arnego, Narla, Salas	Navia, Tambre, Ulla, Miño, Limia	Habitat assessment and management of host populations	

5.4 Discussion

Documenting the spatial extent and the importance of biotic interactions affecting species abundance can benefit conservation practice. Abundance predictions showed areas where biotic interactions between mussels and fish are still occurring, helping to identify conservation strategies for preserving biotic interactions at multiple locations. Assessment of population structure in areas with high mussel abundance helped to discern areas where mussel populations are still recruiting with successful reproduction and parasitism. There is a general lack of laws and regulations concerning conservation that typically fail to consider the interactions of species with strong biophysical linkages (Soulé *et al.,* 2005). This novel two-step approach that combines abundance prediction from spatial models of biotic interactions along with population structure assessment can be applied in other freshwater species to define conservation strategies for interacting groups.

Ecological Insights and Biotic Interactions

Low abundances were predicted in upstream headwater areas, and intermediate to high abundance predictions occurred in spatially limited areas within the study region (Figure 5.8). This fact coincides with the predictions of low and high probability of presence obtained in for MaxEnt predictions (Figure 3.2). These specific areas of high probability of presence and high abundance predictions suggest that at least some contemporary habitats of *M. margaritifera* provide a stable physical structure for mussel beds (see Chapter 3 for an ecological discussion). Moreover, analysing the differences at a smaller scale between the results of high of probability of mussel presence (Chapter 3) and mussel abundance model predictions, it is notable that some areas of high probability of presence coincides with areas of intermediate predicted abundance. As in this case study there is a clear biophysical linkage between the mussel and its host fish, this variability in abundance predictions in the areas where the species is present may be caused by a decrease in the interactions between mussels and fish biotic interactions or by other variables not included in the model such as human impacts. However, the analysis of age profiles combined with the

abundance predictions still helps to identify areas where human impacts may be controlling the mussel abundance patterns, providing information to delimit conservation zones. This idea will be further developed in the section of conservation in this discussion.

Regarding biotic interactions, elsewhere, extinction of mussels from several river systems and upstream of dams has been linked to loss of appropriate host fish (Kat & Davis, 1984; Watters, 1996). The models analysed and presented in Chapter 4 clearly showed that fragmentation of many rivers by dams at a regional scale is an important source of reduced mussel abundance. Dams have fragmented most rivers in the study area (Hervella & Caballero, 1999; World Commission on Dams, 2000) and they have excluded the Atlantic salmon and migratory trout from much of their former range in the northwest portion of the Iberian Peninsula (Hervella & Caballero, 2002; Caballero *et al.,* 2006; García de Leaniz, 2008).

In previous chapter, the difference in variance explained by the domains or stratifications suggested an important biophysical control of migratory host fish on mussel abundance (Chapter 3). Movements and dispersal of migratory host fishes appear to be crucial factors affecting mussel densities in areas downstream of dams in this study region. The model results suggest that presence of migratory salmonids is a key factor contributing positively to species abundance. These results indicate strong biophysical linkages between the parasite and its hosts and they especially point to the negative effects of dams on river ecosystems that affect both species so their effects should be considered when defining conservation zones.

Conservation Zonation to Protect Biotic Interactions

The analyses and predictions of abundance helped to assess the importance of biotic interactions in the study region. These results showed spatial autocorrelation was apparent out to 17 km and was strongest at distances less than 10 km (Figure 4.5). Thus, assessment at a 10 km scale could easily identify river segments that are higher or lower in species abundance, for instance. In a different perspective, the results

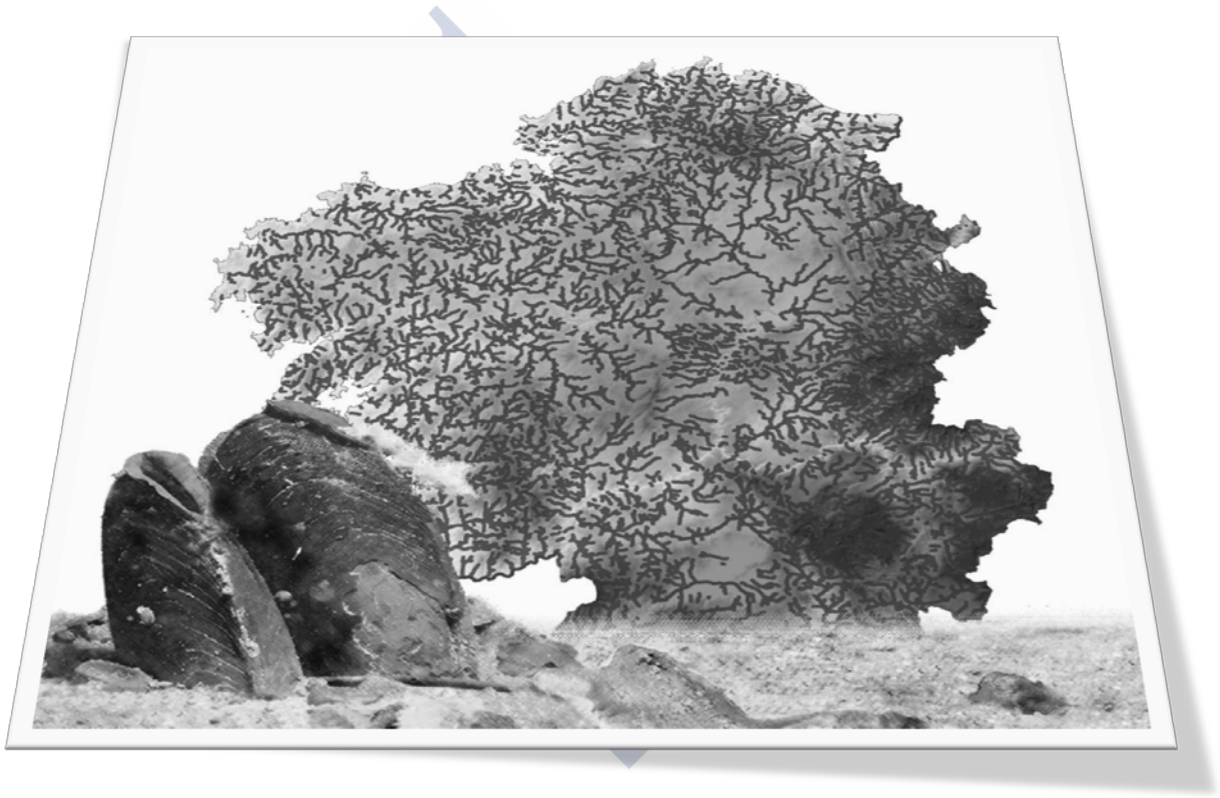
suggest 17 km as a minimum river segment length for restoring or managing biotic interactions and for conducting sampling strategies (Isaak *et al.,* 2014; Som *et al.*, 2014) in this case focused on biotic interactions.

Here, combining abundance predictions and demographic age profiles helped to define four categories that represent conservation zones, where common conservation strategies can be applied to multiple populations of mussels and their host fish. Areas where biotic interactions are still occurring should be protected and monitored because they lead to the presence of at least 20% of the population being 5 to 10 years of age, especially within zones where migratory hosts are still present (River Tea and Eo) (Figure 5.9). Moreover, in the River Eo it is known that a number of sampling sites, not included in this study, also have more than 20% of individuals of 5 to 10 (Outeiro *et al.*, 2008). From the available data, River Eo contains the best-preserved *M. margaritifera* populations in Galicia and a future benchmark for restoration of other mussel populations. In contrast, for the remainder of the zones defined here, conservationists could explore ways to concurrently manage host fish and mussels because recruitment of mussels may be regulated by (a) the density of both mussels and fish (Haag & Warren, 1998; Strayer *et al.,* 2004; Arvidsson *et al.,* 2012), by the relative richness of suitable species of host fish (Schwalb *et al.,* 2011; Douda *et al.,* 2012) or by habitat qualities that affect parasite and/or host fish species. Moreover, the existence of a few mussel populations with recruitment upstream of dams where migratory salmonids are excluded (river Limia) (Figure 5.9) offer hope that management strategies can aim at ensuring adequate numbers of juvenile host fish are available for parasitism by the mussel. For example, an agency might introduce juvenile trout or salmon in association with mussels to facilitate parasitism or impose fishing regulations to protect host fish. Moreover, as dams have reduced and altered the available habitats for young fish and mussels, more effort is needed to simultaneously restore habitats for host fish and mussel populations. Furthermore, the existence of mussel populations without recruitment where migratory host fish are present may indicate that human impacts may be causing lack of mussel recruitment (Rivers Masma, Ouro, Mandeo and Landro) (Figure 5.9). Finally, in the study region

there are zones where migratory fish are absent and there is a lack of mussel recruitment, where management actions regarding habitat assessment and restoration and host fish could be implemented. This work will help to improve the future revision of the recovery plan (Ondina *et al.,* 2010) for *M. margaritifera* in Galicia.

This work highlights the fact that biodiversity conservation will benefit from broad-scale comprehensive studies to understand biotic interactions and how they influence species and their affiliates. Having such knowledge will help to design integrative conservation management for different regions. Conservation efforts that promote recovery of ecosystem processes can facilitate recovery of biotic interactions between species and thereby improve conditions for multiple imperilled species at the same time.

6. PILOT SURVEY OF HABITAT CHARACTERISTICS OF THE *M. margaritifera* POPULATIONS IN THE STUDY AREA



”Noventa e nove non son un cento”

USC
UNIVERSIDADE
DE SANTIAGO
DE COMPOSTELA

6 PILOT SURVEY OF HABITAT CHARACTERISTICS OF THE *M. margaritifera* POPULATIONS IN THE STUDY AREA

6.1. Introduction

Understanding habitat characteristics is necessary for the implementation of endangered species recovery and habitat restoration. The freshwater pearl mussel is endangered within its range and it is protected under national legislation of several countries in Europe as well as by the EC Habitats Directive (Council Directive 92/43/EEC), which requires Special Areas of Conservation to be designated to safeguard this species. Moreover, the presence of a recruiting population of *M. margaritifera* is considered a sign of a healthy functioning river (Geist, 2010). As this species occurs in a wide range of catchments in Europe many efforts are being made to characterize their microhabitat characteristics in the different areas of its range (Varandas *et al.*, 2013).

In Galicia there is a lack of data about the microhabitat characteristics of *M. margaritifera* populations. Thus, it is necessary to report the microhabitat conditions of the populations in the rivers so that a baseline is available to assess future changes that might affect the population status. Moreover, in Galicia there are still populations showing recent recruitment (Lois *et al.*, 2014) so it is important to collect data about microhabitat conditions in existing populations. This will provide data about the reference conditions of habitat characteristics of this area. In this chapter a pilot study reports the first assessment of the habitat characteristics found in the rivers of Galicia. This pilot work will serve as guide to design future sampling and monitoring strategies of microhabitat characteristics for freshwater pearl mussel in Galicia.

6.2. Materials and Methods

Microhabitat characteristics were measured in 13 different rivers belonging to 11 basins (Figure 6.1). The measurements were carried out in 50 m transects described in Chapter 2 (50 meter transects in stratified sampling, phase II). The criteria used to select the sampling sites for this pilot survey were:

- Select sampling locations with high densities (see Chapter 2)
- Include sampling points in different basins.
- Include locations where recent recruitment was recorded.
- The 13 sampling locations selected are shown in Figure 6.1 below.

River Substrate

Substrate characteristics, such us texture analyses and chemical analyses such as redox gradients and penetration resistance of the substratum, were measured following the methods described in Geist & Auerswald (2007). The measurements were carried out during summer conditions when water levels were low. A representative stream substratum sample was collected at each sampling site. Samples were sieved in decreasing mesh sizes (20, 6.3, 3.15, 2.0, 1.0, 0.63, 0.2 and 0.1 mm). A redox probe with a platinum electrode and a reference soltion ($Ag/AgCl_2$) was used to measure redox potential (Eh) in stream water and at 5 cm and 10 cm substratum depths to assess the effect of reduction of oxygen content of the interstitial water (Figure 6.1). Redox potential was measured at one transect in each of 13 rivers (135 spots per stream transects). This parameter has also been reported for several *M. margaritifera* populations elsewhere in Europe where values below Eh 300 mV indicated anoxia (chemically reducing conditions) and values above 300 mV indicated oxic conditions (Geist & Auerswald, 2007).

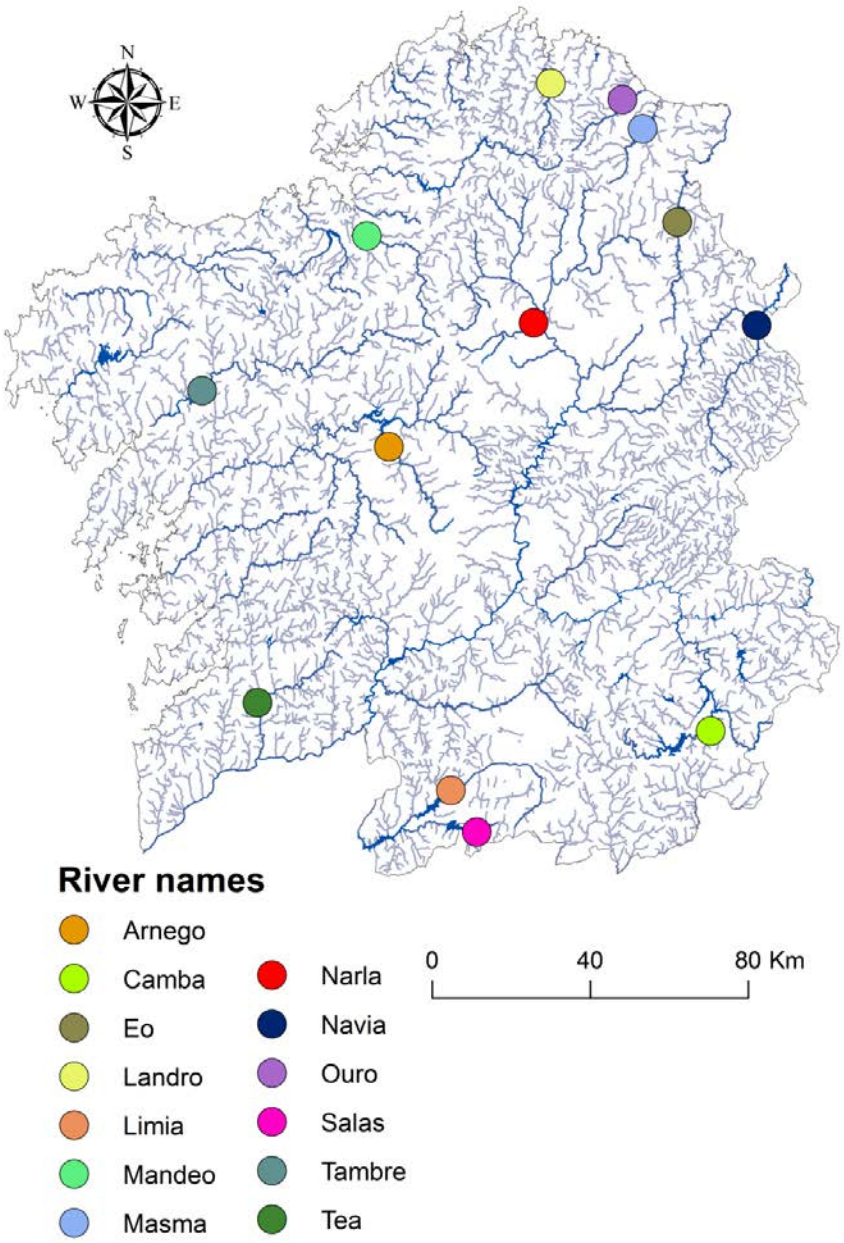


Figure 6.1.- Sampling locations selected to conduct microhabitat measurements.

Resistance of the substratum surface to penetration was measured with a hand-held pocket penetrometer. The penetrometer measures the consolidation of the

riverbed substratum and increasing penetration resistance is consistent with accumulation of fine sediment in the interstial pore spaces of the streambed. For reporting the characteristics of the different stream bed types, four metal adapter discs with diameters of 15, 18, 20 and 25 mm were used. Readings were corrected according to the adapter area as described in Geist & Auerswald (2007). Substratum resistance ($kg.cm^{-2}$) was measured at one transect in each of 13 rivers (135 spots per stream transect).

Water Quality

Water samples were collected seasonally in one-year study that measured parameters including pH, conductivity (Cond. µS/cm), dissolved oxygen (DO mg/l) and percent saturation of dissolved oxygen (DO%). These parameters were measured with a multi-parameter Eutech PC 700. For analysis of dissolved ammonia ($N\text{-}NH_4^+$ mg/l), the colorimetric technique of Blue Indophenol and a spectrophotometer Varian UV/VIS Cary1E were used.The remaining parameters were determined by colorimetric test with a spectrophotometre Oddissey DR-2500. These analyses were carried out by *Instituto de Biodiversidade Agraria e Desenvolvemento Rural* (IBADER) and by *Rede de Infraestructuras de Apoio á Investigación e ó Desenvolvemento Tecnolóxico* (RIAIDT) from Universidade de Santiago de Compostela (Campus Lugo).

6.3. Results

Results from microhabitat analyses are presented for each site within the 13 rivers analyzed in separate sheets below (Figure 6.3). Variables concerning river substratum granulometry (in % on pie chart), redox potential (Eh), penetration resistance (kg/cm^2) each 10 meters along with information about the age frequency distribution for each site displayed in the graphs below. The physicochemical parameters, the percent of different age classes at each site and the mussel density in each site is presented in tables within the figure for each site. The sites are displayed

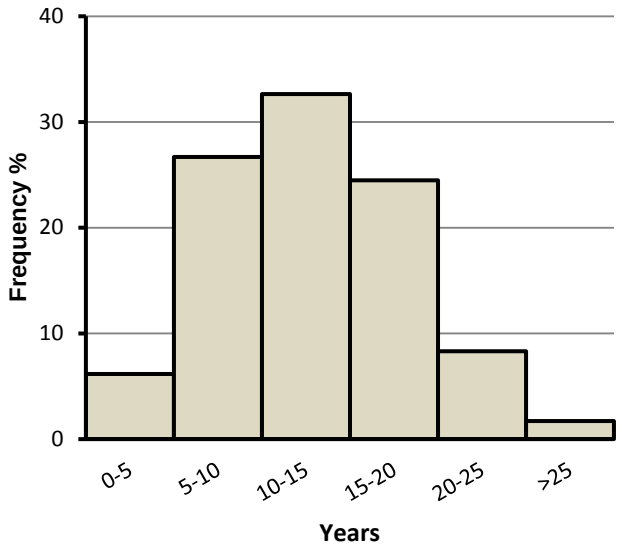
and organized in code colors; green for sites that had recent recruitment in the last 5 years (Limia, Tea, Mandeo and Ouro) and blue for the rest of them.

Figure 6.2.- Photos of microhabitat data collection in the field. Photos by S. Lois, A. Outeiro and P. Ondina

Figure 6.3.- Results from microhabitat analyses and water quality papameters; are presented for each site within the 13 rivers analyzed in separate sheets below. Variables concerning river substratum granulometry (in % on pie chart), redox potential (Eh), penetration resistance (kg.cm^{-2}) each 10 meters along with information about the age frequency distribution and mussel density. The cumulative percent of a population is shown for < 5%, <10 % and <15 % years; in addition the % of individulas < 65 mmm is given along with the average population density. Results for each transect are shown below.

LIMIA RIVER

Age frequency distribution



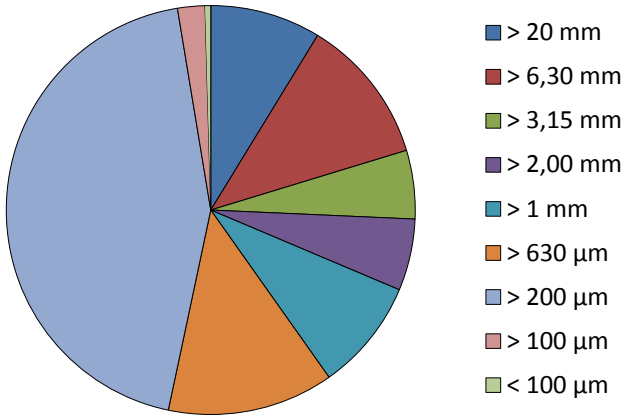
***M. margaritifera* population**

< 5 years %	6.2
<10 years %	32.9
< 15 years %	65.5
< 65 mm %	18.8
Density (ind./m^2)	1.46

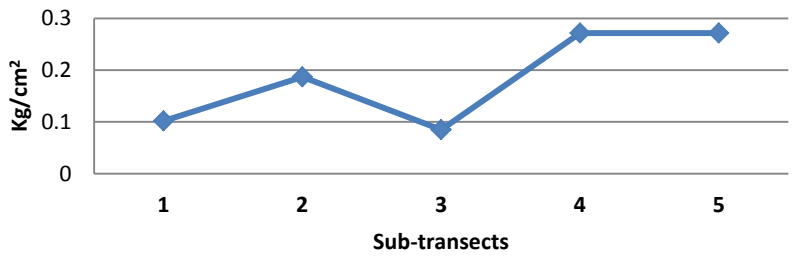
Water quality

pH	6.47
DO (%)	81.53
DO (mg/l)	7.22
Cond (µS/cm)	116.27
DBO5	0.442
PO_4^{3-} (mg/l)	0.084
P total (mg/l)	0.126
$N\text{-}NO^{3-}$ (mg/l)	0.721
$N\text{-}NO^{2-}$ (mg/l)	0.003
$N\text{-}NH^{4+}$ (mg/l)	0.060

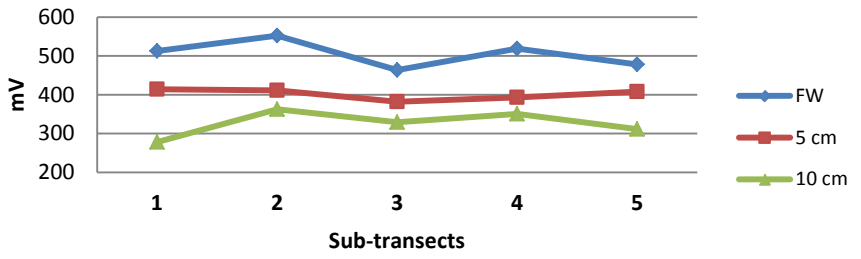
River substratum granulometry (%)



Penetration resistance of the substratum

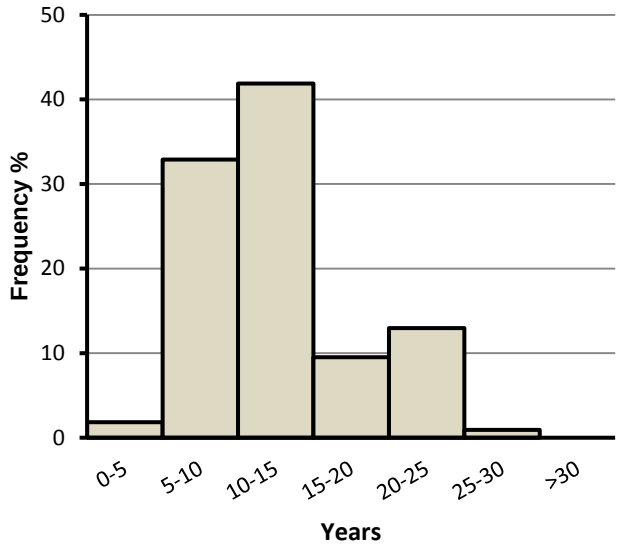


Redox potential (Eh)



TEA RIVER

Age frequency distribution



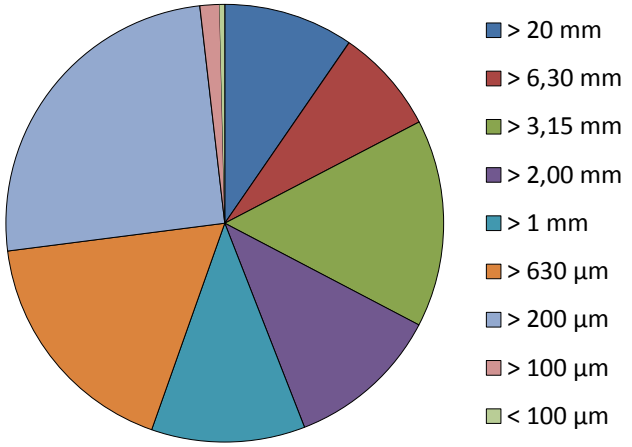
***M. margaritifera* population**

< 5 years %	1.8
<10 years %	34.7
< 15 years %	76.6
< 65 mm %	18.5
Density (ind./m²)	2.50

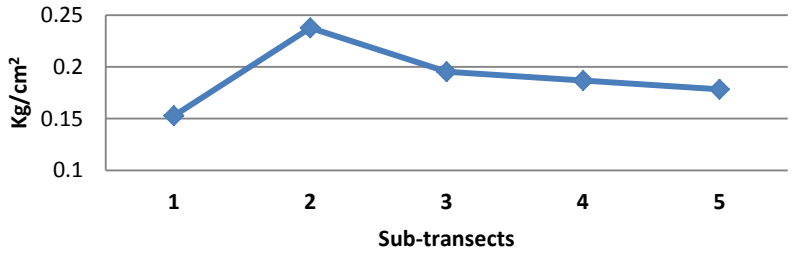
Water quality

pH	6.45
DO (%)	88.46
DO (mg/l)	7.27
Cond (µS/cm)	39.77
DBO5	0.000
PO_4^{3-} (mg/l)	0.031
P total (mg/l)	0.084
$N\text{-}NO^{3-}$ (mg/l)	0.218
$N\text{-}NO^{2-}$ (mg/l)	0.001
$N\text{-}NH^{4+}$ (mg/l)	0.011

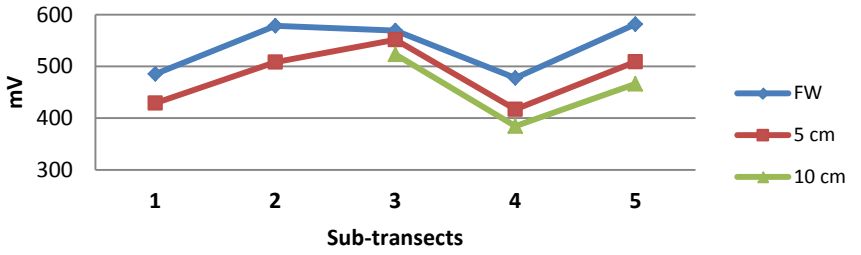
River substratum granulometry (%)



Penetration resistance of the substratum

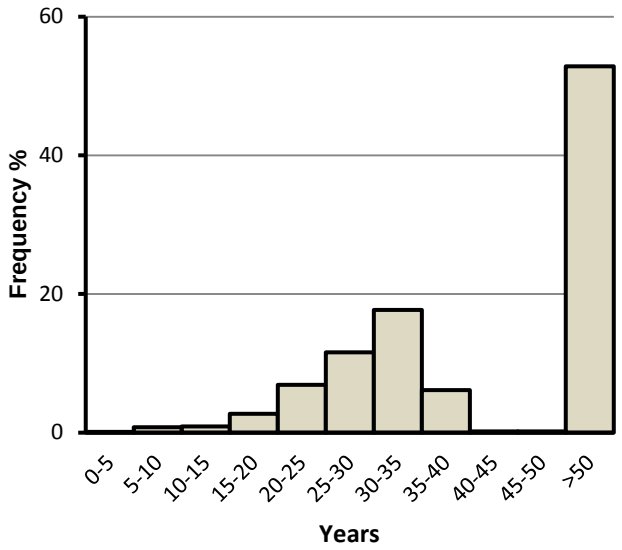


Redox potential (Eh)



MANDEO RIVER

Age frequency distribution



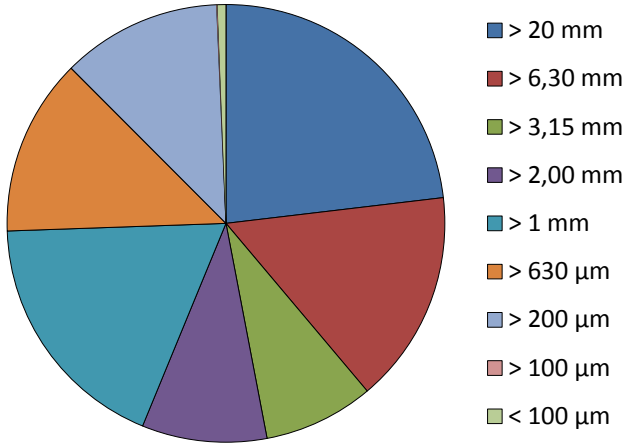
***M. margaritifera* population**

< 5 years %	0.5
< 10 years %	0.9
< 15 years %	6.2
< 65 mm %	13.0
Density (ind./m²)	3.36

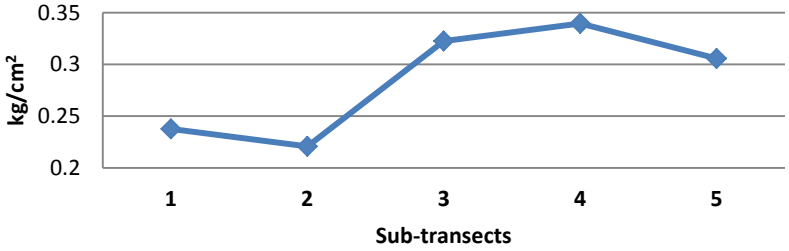
Water quality

pH	6.66
DO (%)	91.81
DO (mg/l)	7.39
Cond (µS/cm)	79.30
DBO5	0.300
PO_4^{3-} (mg/l)	0.033
P total (mg/l)	0.048
$N\text{-}NO^{3-}$ (mg/l)	0.781
$N\text{-}NO^{2-}$ (mg/l)	0.001
$N\text{-}NH^{4+}$ (mg/l)	0.015

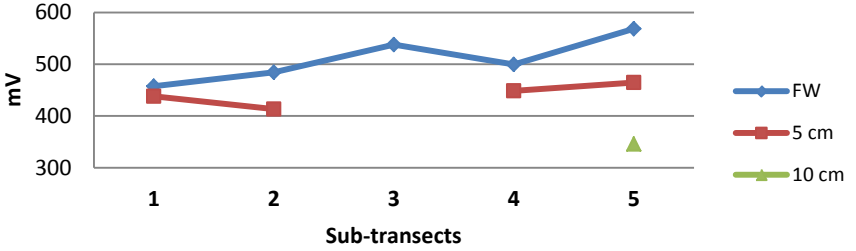
River substratum granulometry (%)



Penetration resistance of the substratum

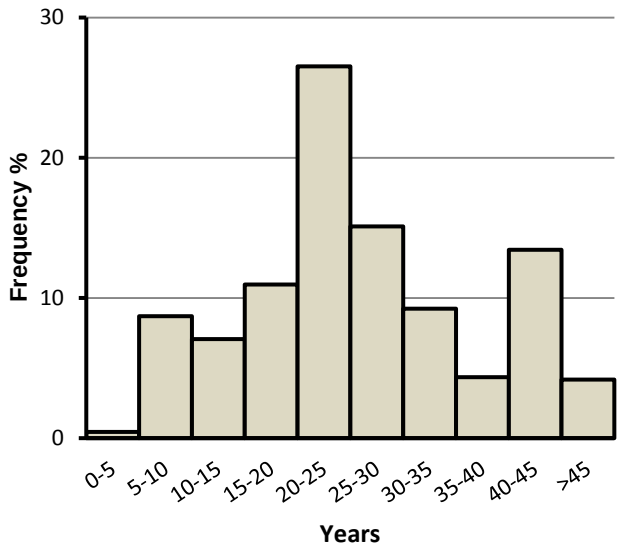


Redox potential (Eh)



OURO RIVER

Age frequency distribution



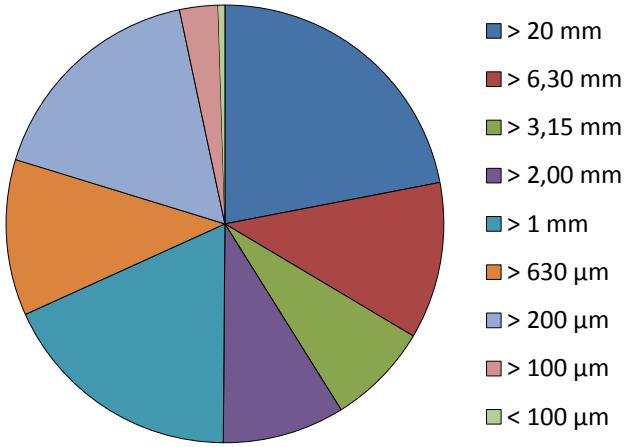
***M. margaritifera* population**

< 5 years %	1.0
< 10 years %	9.0
< 15 years %	18
< 65 mm %	1.4
Density (ind./m²)	6.67

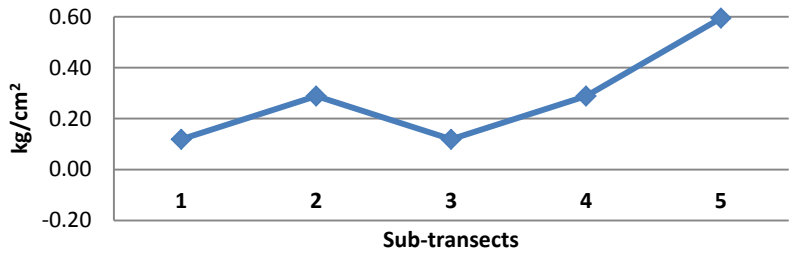
Water quality

pH	6.72
DO (%)	89.27
DO (mg/l)	7.36
Cond (µS/cm)	68.61
DBO5	0.300
PO_4^{3-} (mg/l)	0.053
P total (mg/l)	0.098
$N\text{-}NO^{3-}$ (mg/l)	0.279
$N\text{-}NO^{2-}$ (mg/l)	0.001
$N\text{-}NH^{4+}$ (mg/l)	0.015

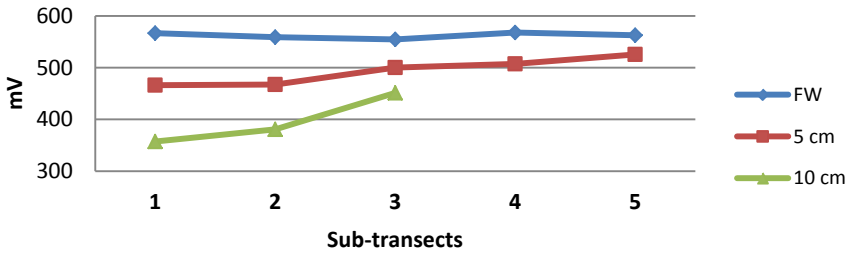
River substratum granulometry (%)



Penetration resistance of the substratum

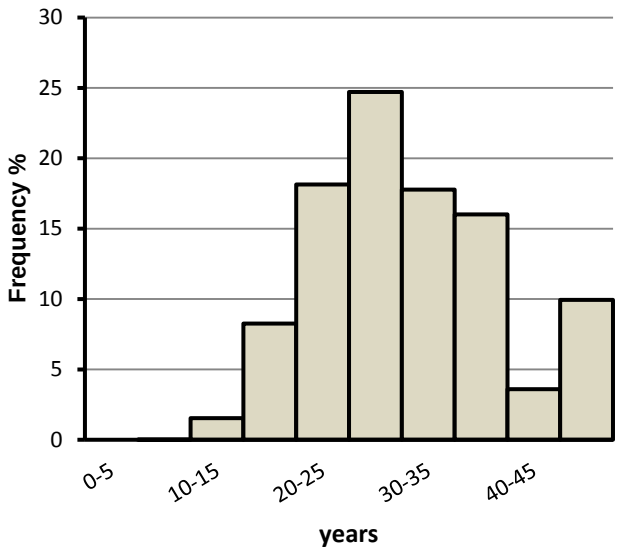


Redox potential (Eh)



ARNEGO RIVER

Age frequency distribution



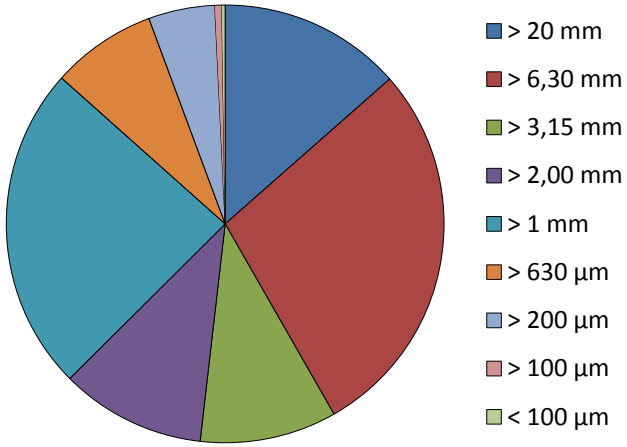
***M. margaritifera* population**

< 5 years %	0.0
< 10 years %	0.0
< 15 years %	1.6
< 65 mm %	0.7
Density (ind./m²)	1.99

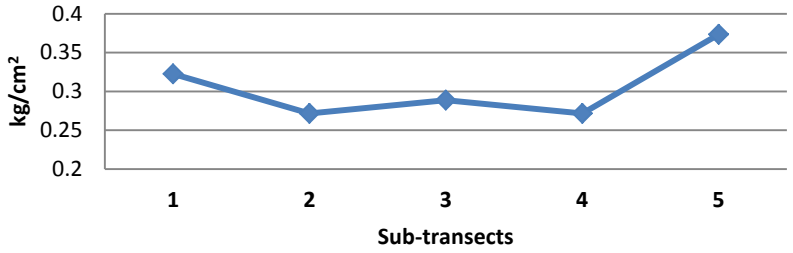
Water quality

pH	6.93
DO (%)	86.91
DO (mg/l)	7.11
Cond (µS/cm)	73.15
DBO5	0.754
PO_4^{3-} (mg/l)	0.044
P total (mg/l)	0.101
$N\text{-}NO^{3-}$ (mg/l)	0.537
$N\text{-}NO^{2-}$ (mg/l)	0.001
$N\text{-}NH^{4+}$ (mg/l)	0.015

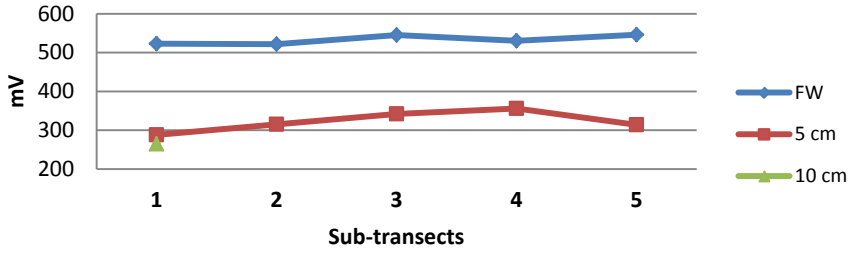
River substratum granulometry (%)



Penetration resistance of the substratum

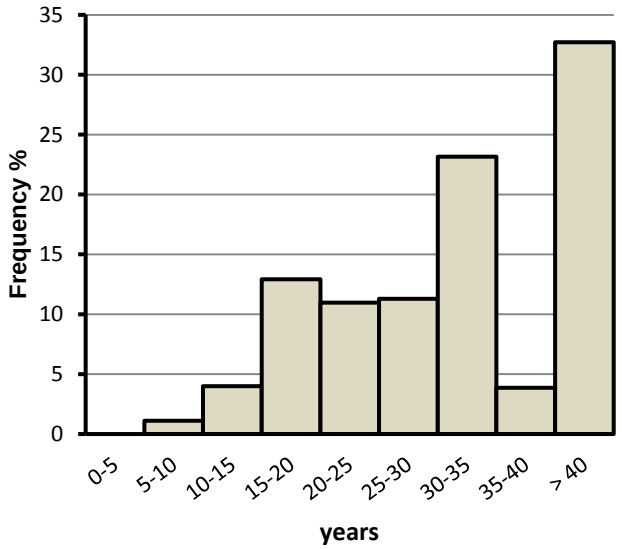


Redox potential (Eh)



CAMBA RIVER

Age frequency distribution



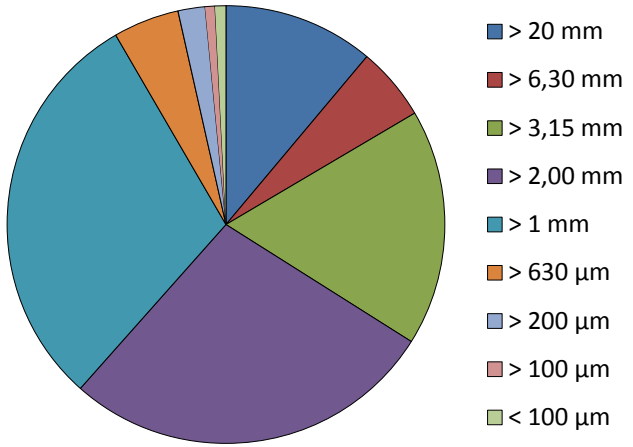
***M. margaritifera* population**

< 5 years %	0.0
< 10 years %	1.0
< 15 years %	5.1
< 65 mm %	0.9
Density (ind./m²)	47.78

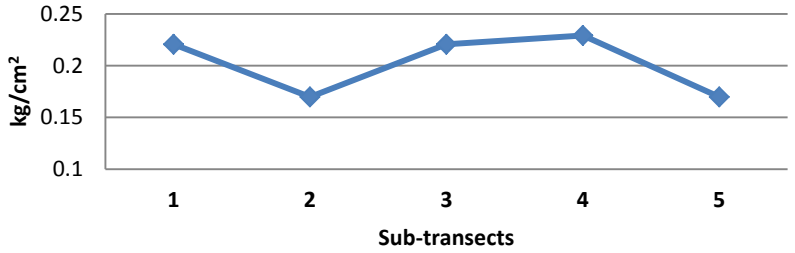
Water quality

pH	6.55
DO (%)	84.65
DO (mg/l)	6.94
Cond (µS/cm)	41.86
DBO5	0.000
PO_4^{3-} (mg/l)	0.048
P total (mg/l)	0.043
$N\text{-}NO^{3-}$ (mg/l)	0.036
$N\text{-}NO^{2-}$ (mg/l)	0.001
$N\text{-}NH^{4+}$ (mg/l)	0.010

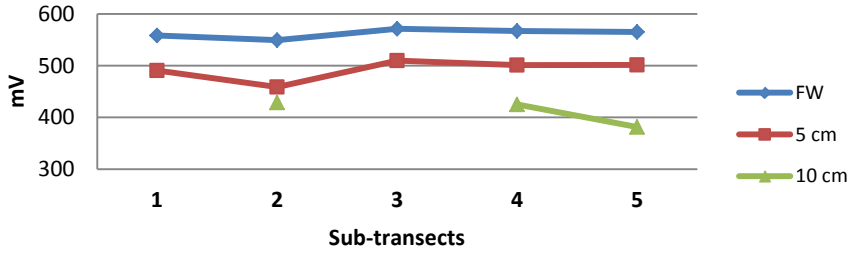
River substratum granulometry (%)



Penetration resistance of the substratum

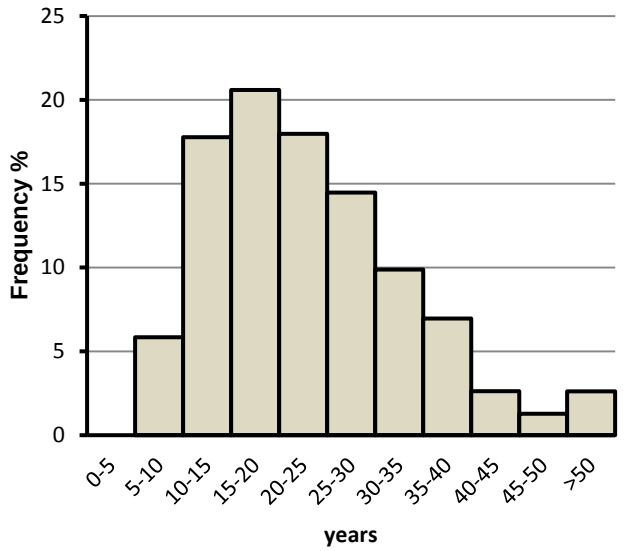


Redox potential (Eh)



EO RIVER

Age frequency distribution



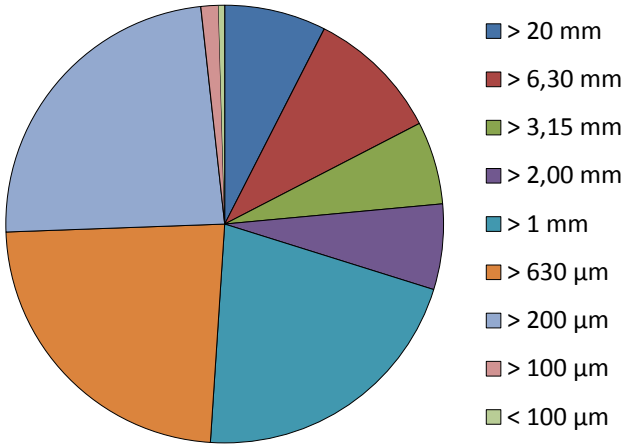
***M. margaritifera* population**

< 5 years %	0.0
< 10 years %	5.8
< 15 years %	23.6
< 65 mm %	11.8
Density (ind./m²)	21.36

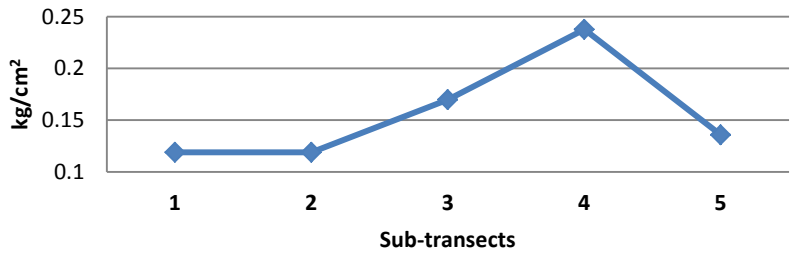
Water quality

pH	6.96
DO (%)	93.29
DO (mg/l)	8.04
Cond (µS/cm)	94.14
DBO5	0.050
PO_4^{3-} (mg/l)	0.037
P total (mg/l)	0.093
$N\text{-}NO^{3-}$ (mg/l)	0.422
$N\text{-}NO^{2-}$ (mg/l)	0.001
$N\text{-}NH^{4+}$ (mg/l)	0.008

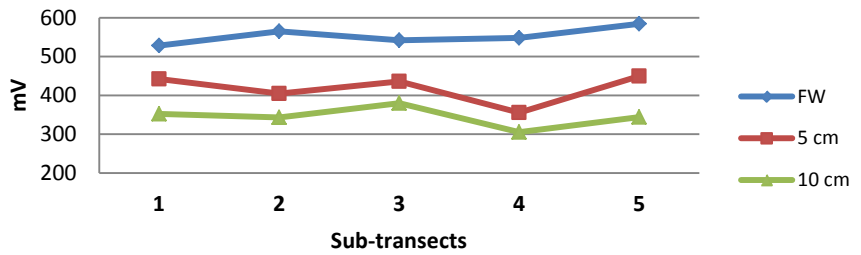
River substratum granulometry (%)



Penetration resistance of the substratum

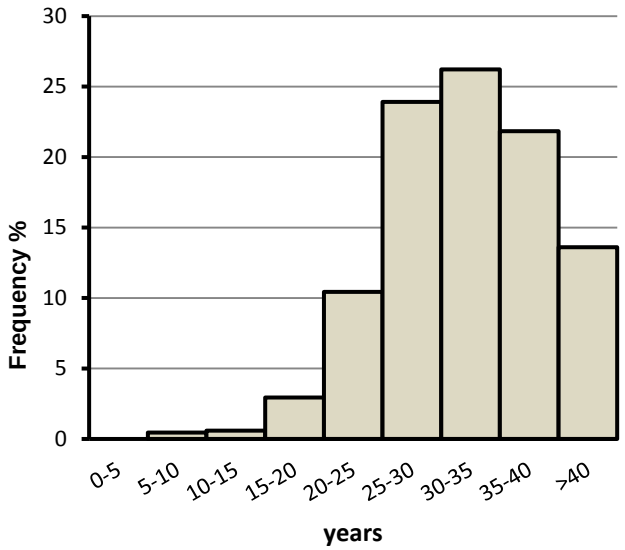


Redox potential (Eh)



LANDRO RIVER

Age frequency distribution



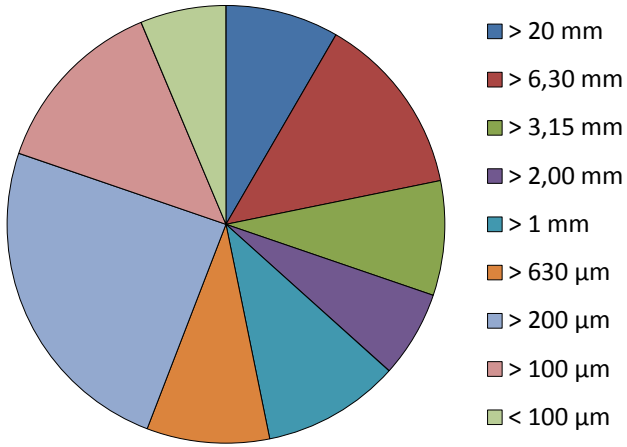
***M. margaritifera* population**

< 5 years %	0.0
< 10 years %	0.5
< 15 years %	1.0
< 65 mm %	0.7
Density (ind./m²)	3.80

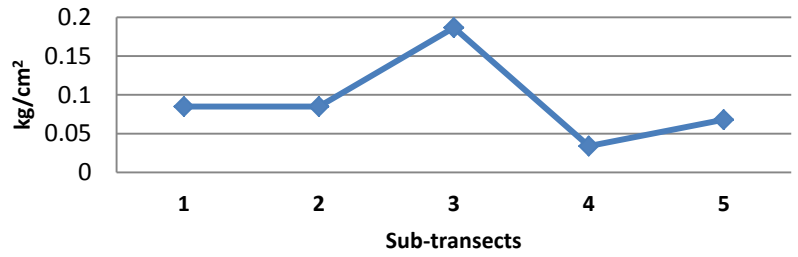
Water quality

pH	6.43
DO (%)	91.34
DO (mg/l)	7.47
Cond (µS/cm)	59.10
DBO5	0.350
PO_4^{3-} (mg/l)	0.030
P total (mg/l)	0.067
$N\text{-}NO^{3-}$ (mg/l)	0.190
$N\text{-}NO^{2-}$ (mg/l)	0.001
$N\text{-}NH^{4+}$ (mg/l)	0.016

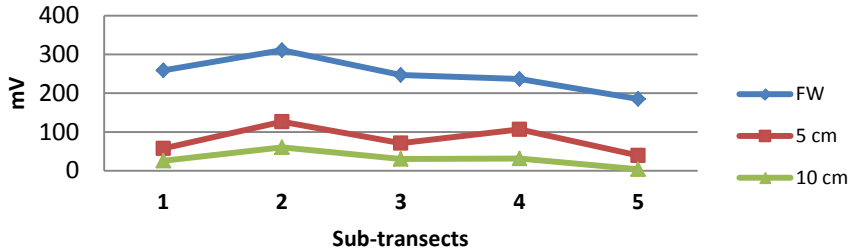
River substratum granulometry (%)



Penetration resistance of the substratum

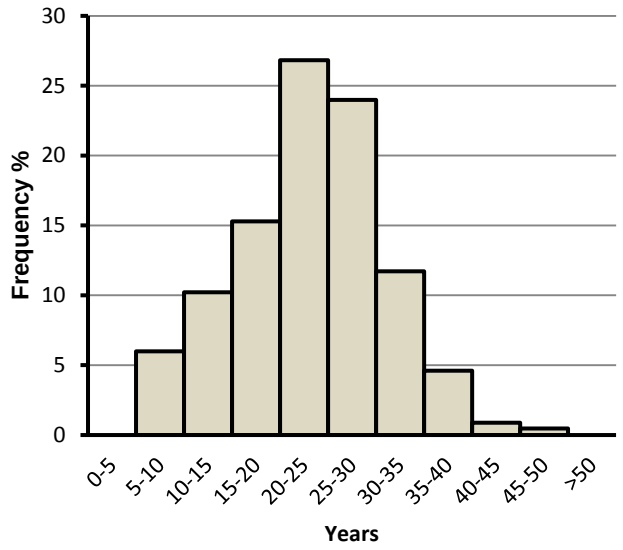


Redox potential (Eh)



MASMA RIVER

Age frequency distribution



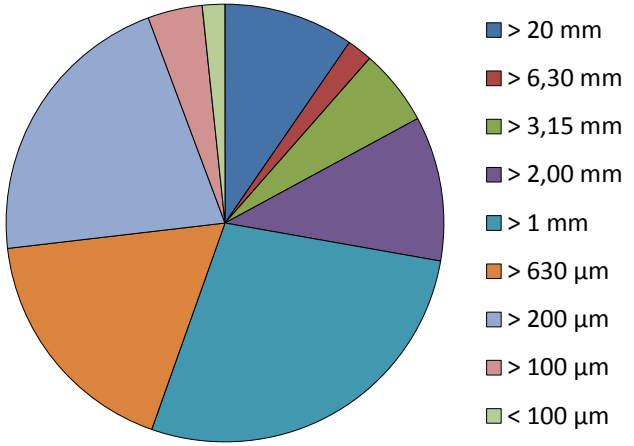
***M. margaritifera* population**

< 5 years %	0.0
< 10 years %	6.0
< 15 years %	16.2
< 65 mm %	8.6
Density (ind./m²)	1.67

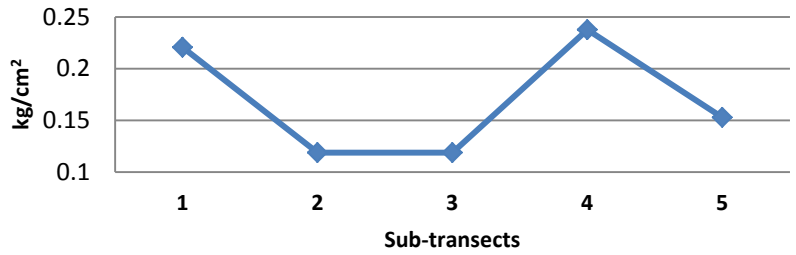
Water quality

pH	7.13
DO (%)	91.91
DO (mg/l)	7.91
Cond (µS/cm)	135.30
DBO5	0.450
PO_4^{3-} (mg/l)	0.130
P total (mg/l)	0.060
$N\text{-}NO^{3-}$ (mg/l)	0.808
$N\text{-}NO^{2-}$ (mg/l)	0.001
$N\text{-}NH^{4+}$ (mg/l)	0.035

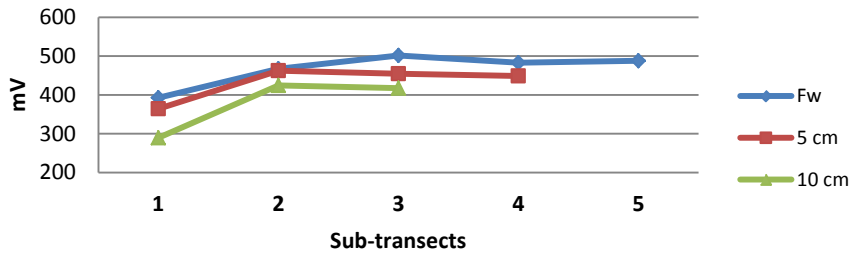
River substratum granulometry (%)



Penetration resistance of the substratum

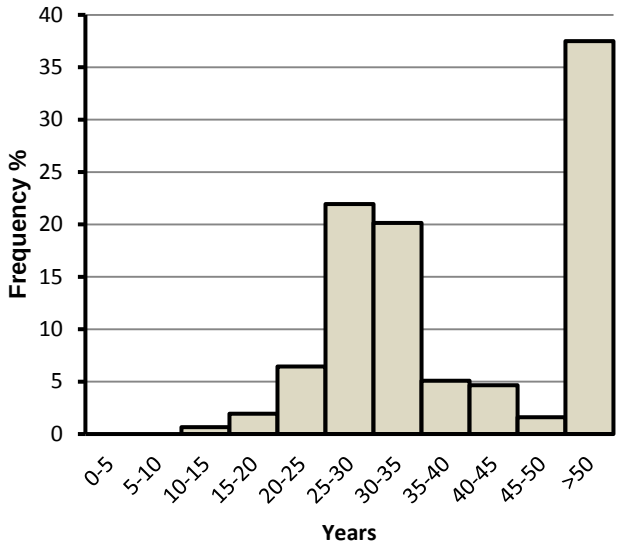


Redox potential (Eh)



NARLA RIVER

Age frequency distribution



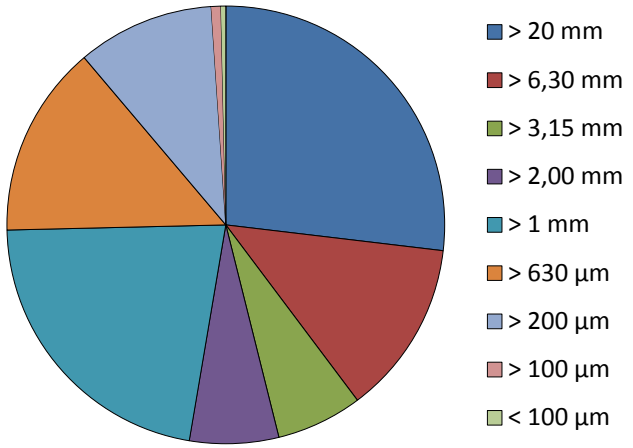
***M. margaritifera* population**

< 5 years %	0.0
< 10 years %	0.0
<15 years %	0.7
< 65 mm %	0.0
Density (ind./m^2)	1.49

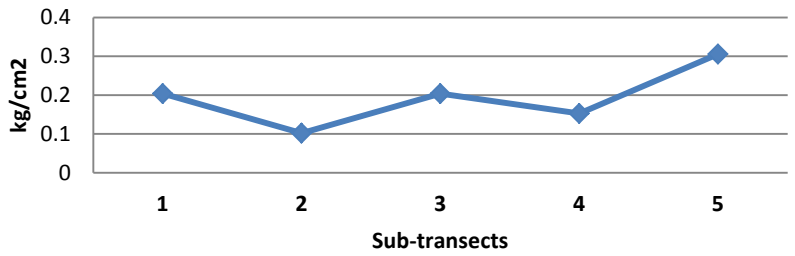
Water quality

pH	6.47
DO (%)	88.77
DO (mg/l)	7.30
Cond (µS/cm)	53.56
DBO5	0.483
PO_4^{3-} (mg/l)	0.070
P total (mg/l)	0.080
N-NO^{3-} (mg/l)	0.318
N- NO^{2-} (mg/l)	0.001
N- NH^{4+} (mg/l)	0.011

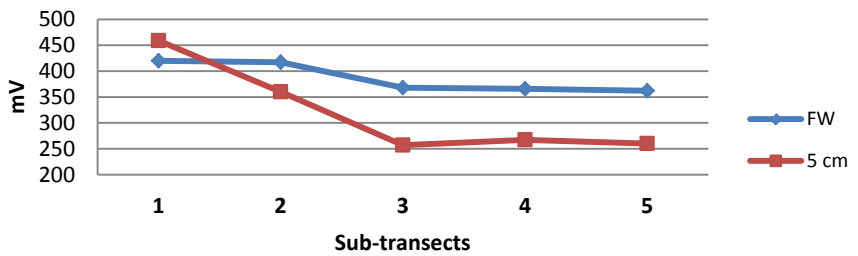
River substratum granulometry (%)



Penetration resistance of the substratum

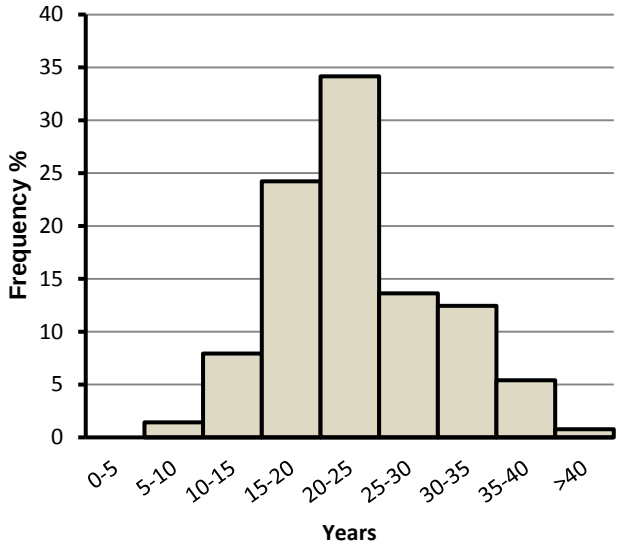


Redox potential (Eh)



NAVIA RIVER

Age frequency distribution



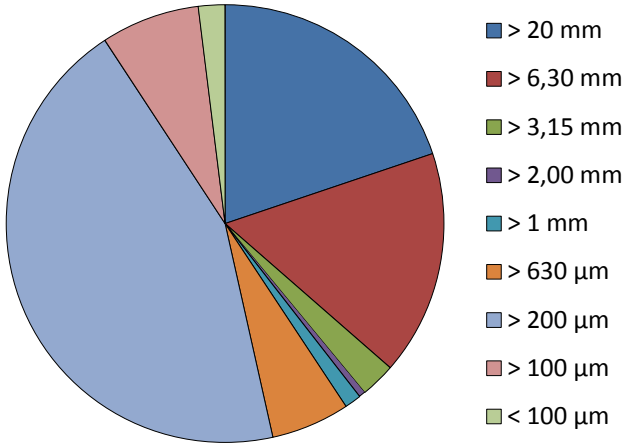
***M. margaritifera* population**

< 5 years %	0.0
< 10 years %	1.4
< 15 years %	9.4
< 65 mm %	5.4
Density (ind./m²)	4.06

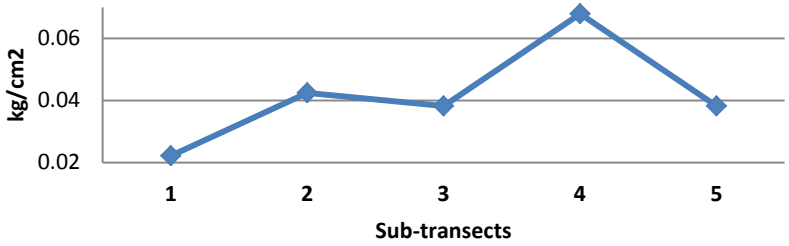
Water quality

pH	7.21
DO (%)	87.88
DO (mg/l)	7.80
Cond (µS/cm)	103.00
DBO5	0.175
PO_4^{3-} (mg/l)	0.034
P total (mg/l)	0.077
$N\text{-}NO^{3-}$ (mg/l)	0.248
$N\text{-}NO^{2-}$ (mg/l)	0.001
$N\text{-}NH^{4+}$ (mg/l)	0.013

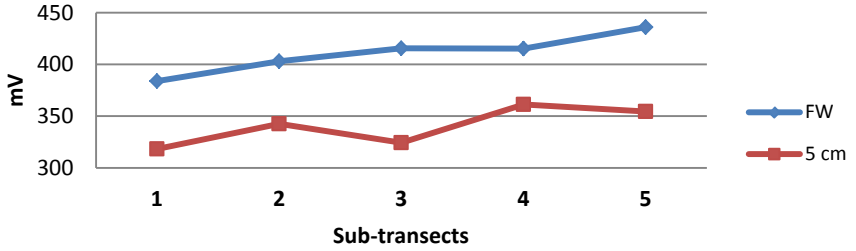
River substratum granulometry (%)



Penetration resistance of the substratum

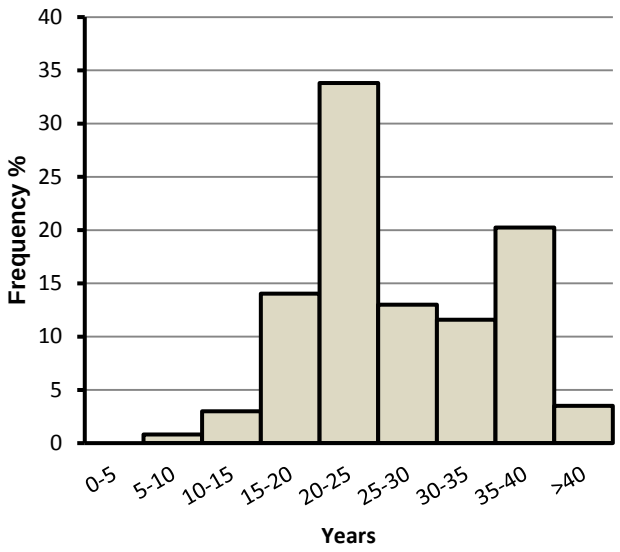


Redox potential (Eh)



SALAS RIVER

Age frequency distribution



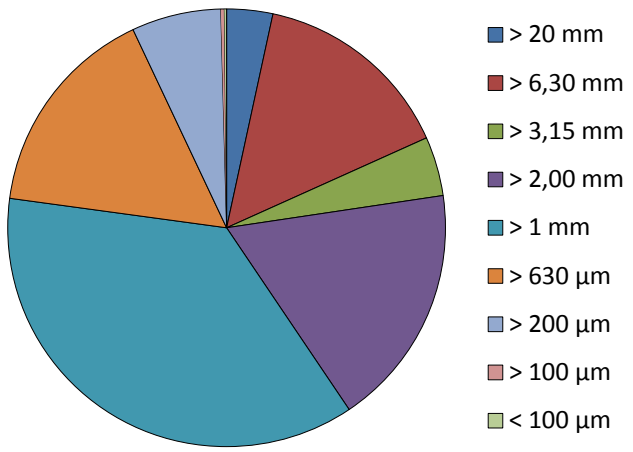
***M. margaritifera* population**

< 5 years %	0.0
< 10 years %	0.8
< 15 years %	3.8
< 65 mm %	10.0
Density (ind./m²)	4.09

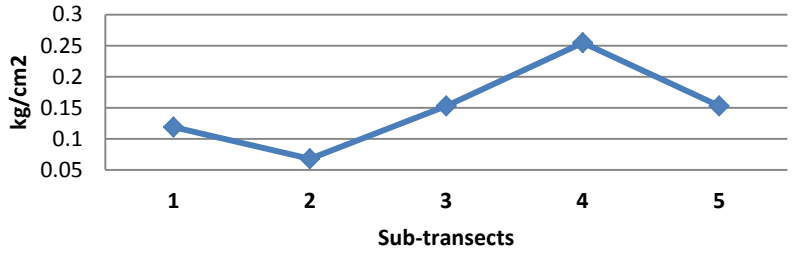
Water quality

pH	6.45
DO (%)	78.97
DO (mg/l)	7.02
Cond (µS/cm)	33.50
DBO5	0.000
PO_4^{3-} (mg/l)	0.053
P total (mg/l)	0.093
$N\text{-}NO^{3-}$ (mg/l)	0.028
$N\text{-}NO^{2-}$ (mg/l)	0.001
$N\text{-}NH^{4+}$ (mg/l)	0.011

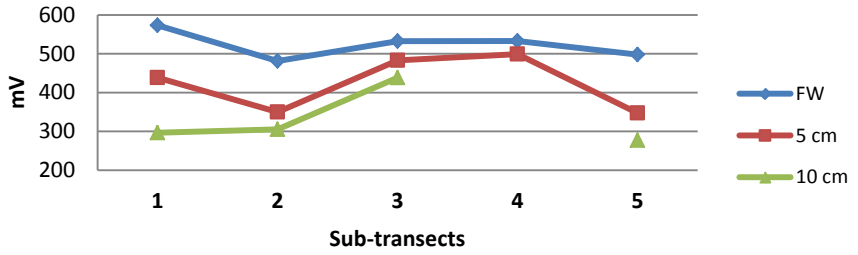
River substratum granulometry (%)



Penetration resistance of the substratum

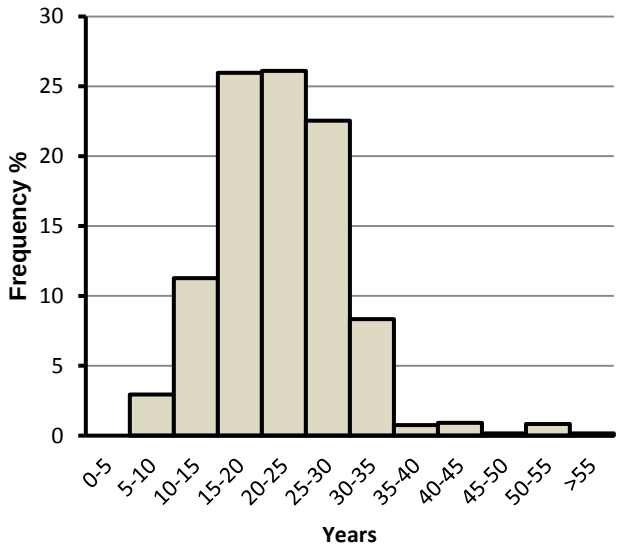


Redox potential (Eh)



TAMBRE RIVER

Age frequency distribution



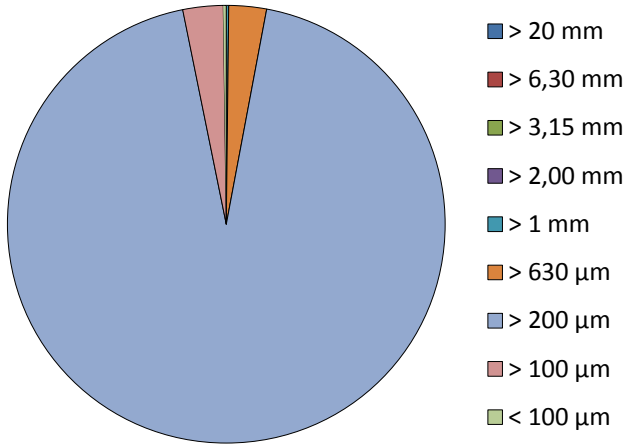
***M. margaritifera* population**

< 5 years %	0.0
< 10 years %	2.9
< 15 years %	14.2
< 65 mm %	3.5
Density (ind./m²)	0.95

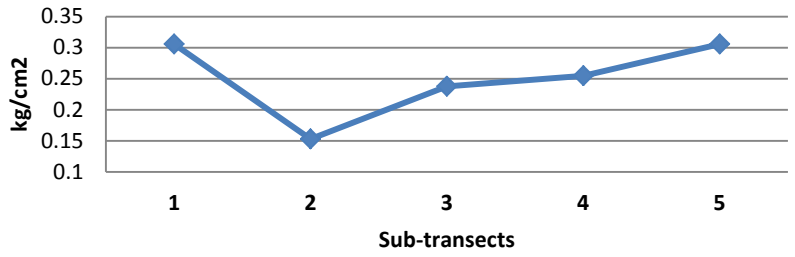
Water quality

pH	6.81
%DO	87.05
DO mg/l	7.27
Cond (µS/cm)	94.88
DBO5	0.325
mg/l PO43-	0.118
mg/l P total	0.133
mg/ N-NO3-	1.142
mg/l N- NO2-	0.002
mg/l N- NH4+	0.032

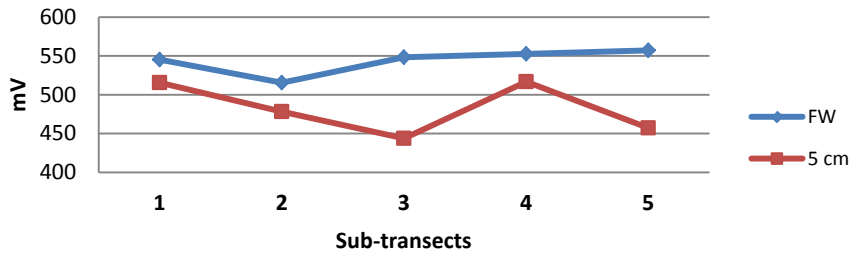
River substratum granulometry (%)



Penetration resistance of the substratum



Redox potential (Eh)



6.4. Discussion

Results from the microhabitat assessments are present for each site within the 13 river analyzed. Sites showed values of DBO 5 less than 1, pH ranged from 6.4 to 7.2, and conductivity ranged from 39.7 to 135.3 µS/cm. These parameters showed similar values as populations of *M. margaritifera* in other areas of its range elsewhere in Europe. For example, Oliver (2000) reported a DBO 5 less than 1.3 and pH values ranging from 6.5 to 7.2. Conductivity values have been reported to vary across the range of *M. margaritifera* [e.g. Finland < 70 (Valovirta, 1995); Central Europe < 200 (Geist *et al.*, 2006) or Portugal < 70 (Reis, 2003). Regarding phosphate levels have showed similar values as the ones found in Portugal (Teixeira *et al.*, 2010). For a review of the habitat conditions reported in Eurpean populations of *M. margaritifera* see Varandas *et al.* (2013).

Regarding measurement of redox potential, the majority of the sites studied showed values higher than 300 mV except Landro and Arnego, indicating well-oxygenated conditions for functional mussel populations (Geist & Auerswald, 2007). Rivers Landro and Arnego showed values lower than 300 mV indicating anoxic conditions not suitable for juvenile mussels probably related with eutrophication conditions. During the data collection for redox potential, several sites had less than 10 cm substrate sediment depth and the deeper measurements of redox potential could not be made.

In general there was no clear pattern in the parameters measured that helped to discern clear differences between sites with and without recruitment, and the parameters studied showed the same values same patterns as the ones described for recruiting populations in other areas of its range (Varandas *et. al*, 2013). However the aim of this chapter was to make a pilot at one site in each river to guide future sampling rather than compare *M. margaritifera* populations. The data collected are not enough to make any conclusions. Thus, any comparison between populations

should be taken carefully. In spite of this shortcoming, the results strengthen the conclusions about the importance of host fish that were described in earlier chapters.

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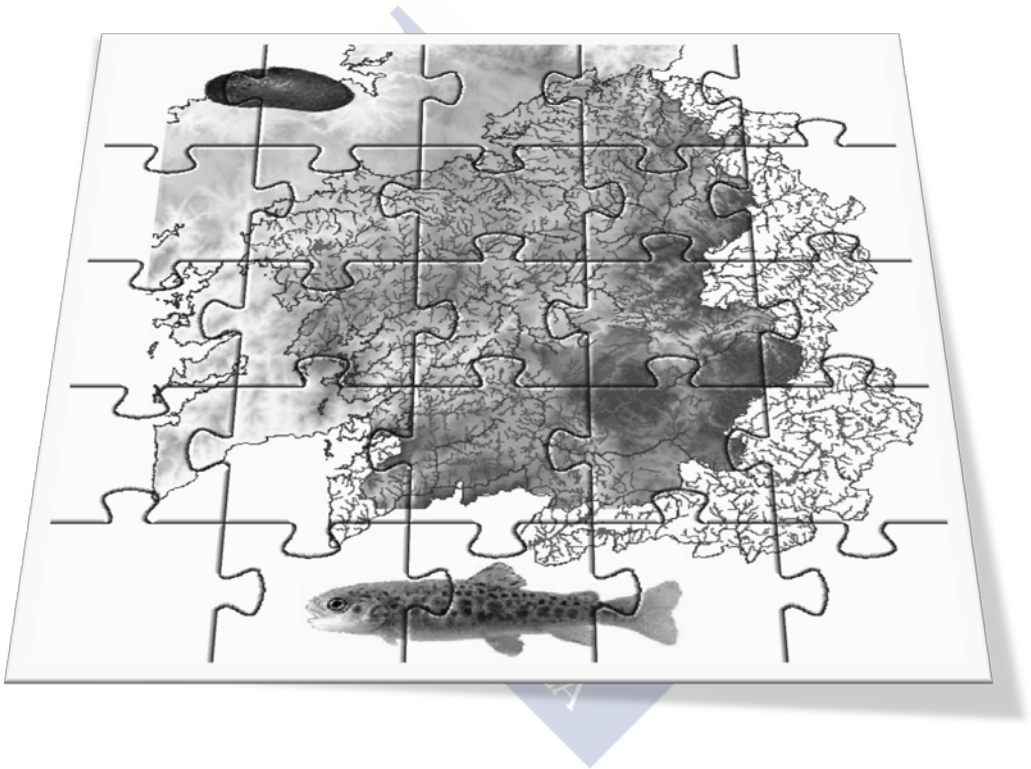
7. CONCLUSIONS



”Moitos poucos fan un moito”

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7. CONCLUSIONS

There is a general need to provide integrative studies to understand, retain and simplify the information contained in spatial patterns of biodiversity in complex ecosystems such as rivers. The objective of this work was to illustrate a novel approach that includes sampling, modelling and predicting for a freshwater species in riverine ecosystems from a broad-scale perspective. The approach was focused on biotic interactions by using *M. margaritifera* in Galicia to gain biological and ecological knowledge to manage its conservation. The conclusions of this work are:

1 Populations of *M. margaritifera* have been found in 54 rivers in 23 drainage basins of Galicia, 27 of which had not been documented before, and information about 522 new localities with presence of the species has been obtained and abundances have been estimated.

2 Galicia has the highest number of populations of *M. margaritifera* within the Iberian Peninsula and apparent recruitment (presence of individuals < 65 mm) was observed in 11 Galician rivers. In contrast age profiles showed that only four sites had more than 20% of the individuals between 5 and 10 years of age.

3 The effects of biotic interactions between M. margaritifera and its hosts are discernible at a regional scale when modelling its distribution in the study area and they are perceptible in model predictions at a local scale. Including biotic interactions in the species distribution models (SDMs) improves the model performance, and host fish variables contributed more than half of the prediction of probability of mussel presence.

4 Salmonid populations and precipitation are key factors controlling the distribution M. margaritifera in the study area. The mapped predictions of probability of presence indicate areas needing further sampling efforts and research to quantify habitat quality. Further, they provide a tool for assessing causes of species absence.

5 The difference in variance explained by two models (path analyses vs geostatistical mixed model) indicated the necessity of accounting for different sources of spatial autocorrelation when modelling mussel abundance in river ecosystems. For the spatial mixed model of mussel abundance, host fish biomass and abundance were the only significant predictors across the study region.

6 The use of functions that account for flow-related processes in river ecosystems (tail-up and tail-down) helped to identify information regarding biotic interactions when modelling abundance. The tail-down component was an important part of spatial autocorrelation of mussel abundance, which links upstream dispersal of the mussel by its host fish. This may indicate the conditions, earlier in time, where upstream mussel dispersal by parasitized host fish occurred into flow-unconnected tributaries.

7 Spatial autocorrelation between mussels and host fish was apparent between sites separated by < 17 km. The spatial mixed model approach helps to estimate different process-based contributions to spatial autocorrelation and it provides an answer to an important question on the spatial extent of biotic interactions on species abundance.

8 More variance in mussel abundance was explained where migratory fish were present. Thus, dispersal biology of host fish appears to be important for mussel dispersal and maintenance of mussel abundance. Fragmentation of many rivers by dams is an important source of reduced mussel abundance. The model results suggest that presence of migratory salmonids is a key factor contributing positively to mussel abundance.

9 Abundance predictions help to identify areas where biotic interactions between mussels and fish are still occurring. Low abundances were predicted in upstream areas and intermediate to high abundance predictions occurred in spatially limited areas within the study region. The geostatistical range, around 17 Km, indicates a useful river segment length for assessing, restoring or managing

biotic interactions between mussels and hosts, and it will serve as a useful scale to design future sampling surveys based on biotic interactions in the study area.

10 Assessment of population age structure in areas with high mussel abundance helped to discern areas where mussel populations are still recruiting. Combining abundance predictions, demographic age profiles and the presence of migratory hosts helped to define conservation zones. Areas where the presence of at least 20% of the population are individuals 5 to 10 years, especially within zones where migratory hosts are still present, should be protected and monitored. In contrast, the remainder of the zones defined areas where conservationists should explore ways to concurrently manage host fish and mussels. The existence of a few mussel populations with recruitment upstream of dams where migratory salmonids are excluded offer hope that management strategies can aim at ensuring adequate numbers of juvenile host fish are available for parasitism by the mussel. Furthermore, the existence of mussel populations without recruitment where migratory hosts are present could indicate that antropogenic impacts are causing lack of mussel recruitment. Finally, for zones in the study region where migratory fish are absent and there is a lack of mussel recruitment, management actions regarding habitat and host fish should be implemented.

11 Microhabitat assessments provided a baseline for future study of the microhabitat characteristics of *M. margaritifera* populations and they will serve as a basis for designing future microhabitat sampling strategies.

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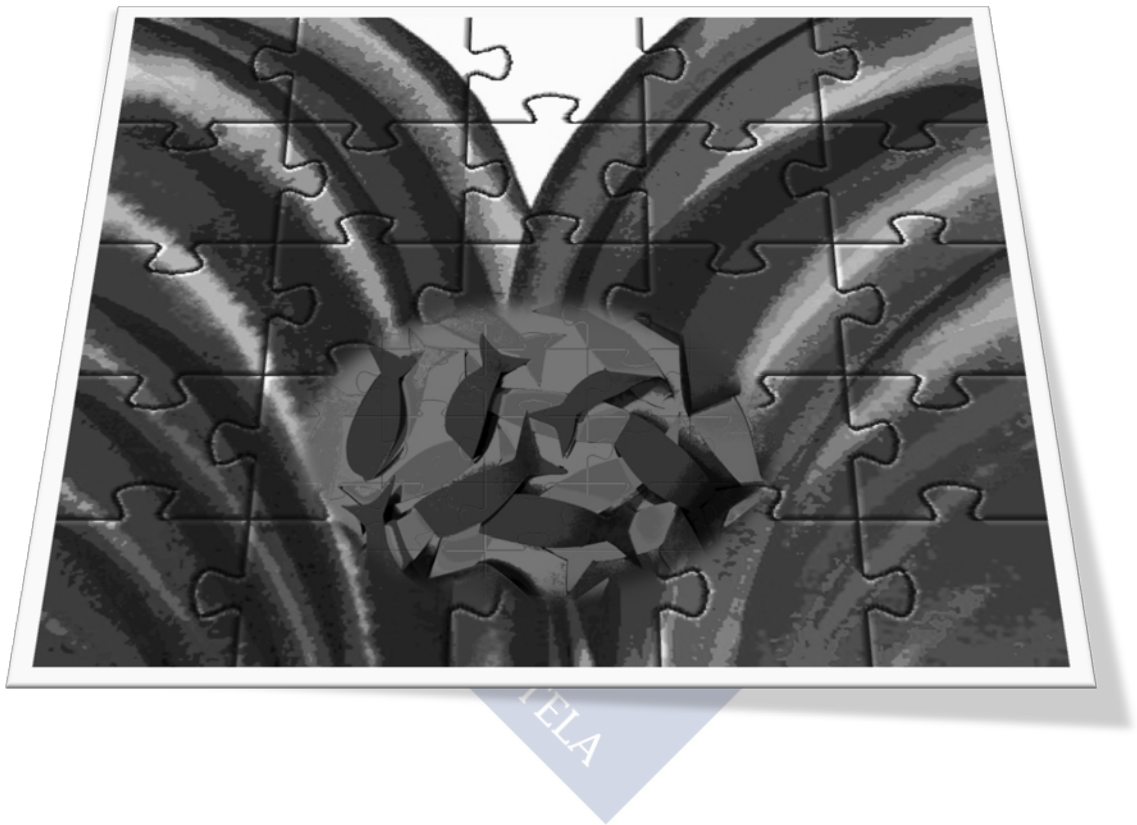
8. RESUMO EN GALEGO



”Ninguén vai ao río que non se molle”

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8. RESUMO EN GALEGO

Os ecosistemas de auga doce son un recurso valioso en termos económicos, culturais, estéticos, científicos e educativos, sendo a súa conservación de interese fundamental para o ser humano. Con todo, e en comparación con outros ecosistemas, están experimentando grandes diminucións na súa biodiversidade (Dudgeon *et al.,* 2006). De aí que exista unha necesidade urxente de achegar un coñecemento, baseado en evidencias (Sutherland *et al.,* 2004), dirixido a entender os seus patróns biodiversidade coa fin de xestionar de forma efectiva a súa conservación (Strayer, 2008).

Entre os ecosistemas de auga doce, os ecosistemas fluviais presentan unha gran complexidade. De forma xeral, son redes de auga integradas na paisaxe, nas que a bacía hidrográfica, ou de drenaxe, é a unidade que delimita os procesos hidrolóxicos nun río. É por iso que, dende a perspectiva da ecoloxía da paisaxe, as bacías son consideradas como illas bioxeográficas (Sepkoski & Rex, 1974; Simberloff, 1974; Minshall *et al.,* 1983; Hugueny, 1989). Así, cada sistema fluvial atópase influenciado polas características específicas da paisaxe da propia cunca, que tamén influirán na súa estrutura e función, tanto a escala rexional (cunca hidrográfica) coma local (microhábitat). Deste xeito, os ríos crean unha rede interconectada de biótopos e gradientes ambientais onde os fluxos de materia e enerxía se producen a través de vías interactivas en catro dimensións diferentes: lonxitudinal, vertical, lateral e temporal (Ward, 1998). Alén do anteriormente mencionado, os diferentes impactos producidos pola actividade humana nos ríos engaden outra fonte de heteroxeneidade espacial.

Por estes motivos, comprender os procesos biolóxicos, físicos, químicos e antropoxénicos que están a ocorrer nos ecosistemas fluviais do planeta é unha tarefa multidisciplinar. Coa fin de abordar esta problemática multidimensional dunha forma holística, definiuse o concepto de paisaxe fluvial ou Riverscape, como o estudo integrado dos procesos que rexen os sistemas fluviais (Schlosser 1991; Fausch *et al.,* 2002; Ward *et al.,* 2002; Allan, 2004). Este termo serve como escala conceptual para o estudo dos patróns de biodiversidade así como dos procesos nos ecosistemas fluviais.

Porén, debido ao desenvolvemento de novas técnicas de análise no marco da ecoloxía da paisaxe e da análise espacial, este concepto está aínda en evolución (Allan, 2004).

Os mexillóns de auga doce son bivalvos da orde Unionoida comunmente chamados náiades. Estímase que existen até 840 especies distribuídas en todos os continentes, excepto na Antártida (Bogan, 2008; Graf & Cummings, 2007). Son filtradores bentónicos, e, polo seu tamaño e as altas biomasas que chegan a alcanzar, exercen no ecosistema fluvial un importante rol no tratamento de partículas, na reciclaxe de nutrientes e na mestura de sedimentos (Spooner & Vaughn, 2008; Vaughn *et al.,* 2008). Por conseguinte, a súa conservación aporta beneficios importantes tanto para a xestión da calidade da auga, como para o mantemento das funcións dos ecosistemas nos que habitan. Alén disto, a súa dependencia dun hóspede móbil, tanto para a supervivencia da fase larval parasitaria como para a súa dispersión ao longo da rede fluvial, convérteas en especies de significativo interese para o estudo das interaccións bióticas e da conectividade fluvial. Con todo, son, probablemente, un dos grupos de animais máis ameazados (Williams *et al.*, 1993; Bogan, 1993, 2008; Strayer *et al*., 2004), polo que existe a necesidade de desenvolver estudos máis completos a gran escala para comprender os factores que controlan as poboacións de mexillóns (Strayer, 2008) e, polo tanto, para deseñar accións de conservación e xestión en diferentes áreas.

É amplamente recoñecido que a conservación da biodiversidade vese facilitada polo mantemento das poboacións de especies fortemente interactivas (Soulé *et al.*, 2005). Non obstante, tanto nos ecosistemas acuáticos coma nos terrestres, existe unha controversia sobre a escala espacial na que estas interaccións son discernibles. A hipótese chamada "*The Eltonian noise hypothesis*" (Soberon & Nakamura, 2009) suxire que as interaccións bióticas poden ter efectos medibles unicamente a pequena escala (Huston, 1999; Pearson & Dawson, 2003). Algúns estudos teñen cuestionado esta idea (Araújo & Luoto, 2007; Heikkinen *et al.*, 2007; Meier *et al.*, 2010; Kissling *et al.*, 2012; Araújo & Rozenfeld, 2014; de Araújo *et al.*, 2014), mentres outros subliñan a necesidade de incrementar o número de exemplos experimentais e ferramentas que

apoien os modelos teóricos (Wisz *et al.*, 2013). Simulacións recentes suxiren que a identificación das interaccións bióticas a calquera escala espacial dependen do tipo de interacción, mostrando que o mutualismo ou o comensalismo tenden a manifestarse a través das escalas (Araújo & Rozenfeld, 2014). Mesmo así, independentemente da dimensión e do tipo de interacción espacial, son escasos os estudos nos que se teñen en conta as interaccións bióticas nos modelos de distribución de especies.

Esta tese describe un proceso completo de mostraxe, modelaxe e predición, enfocado ás interaccións bióticas. Para levar a cabo este obxectivo, o mexillón de auga doce *M. margaritifera* (Linnaeus, 1758) usarase como especie obxecto de estudo. Esta náiade, pertencente á familia Margaritiferidae (orde Unionoida), distribúese polos ríos oligotróficos da rexión Holártica de Europa e América do Norte. Caracterízase polo facto de que a súa fase parasita larval se desenvolve nas galadas dun peixe salmónido, que actúa como hóspede, así como por presentar unha gran lonxevidade podendo acadar os 200 anos de vida (Mutvei & Westermark, 2001). Aínda que é considerada como unha especie chave para a conservación dos ecosistemas acuáticos (Geist, 2010), está ameazada en todo o seu rango de distribución, atopándose incluída na categoría "En Perigo" pola UICN e sendo considerada, ademais, un dos bivalvos de auga doce máis ameazados do mundo (Machordom *et al.*, 2003).

Malia que recentemente se atoparon novas poboacións de *M. margaritifera* nalgunhas zonas de Europa (Ostrovsky & Popov, 2011; Varandas *et al.*, 2013; Cosgrove *et al.*, 2014), a revisión máis actualizada sobre a súa distribución e estado de conservación é aportada por Geist no ano 2010. Nese traballo, o cuadrante noroeste da Península Ibérica aparece como o límite meridional da distribución europea da especie. Neste territorio os escasos datos históricos sobre a súa distribución apuntaban á idea de que os ríos da Comunidade Autónoma de Galicia eran os que presentaban o maior número de rexistros da especie (Macho, 1878). A pesar diso, até agora non se tiña realizado ningún un traballo que tivese como obxectivo coñecer con detalle a distribución desta especie nos ríos de Galicia. Debido a todo o exposto anteriormente, o estudo de *M. margaritifera* nos ríos de Galicia mostra unha clara

oportunidade para afondar no coñecemento sobre as técnicas de mostraxe, modelización e predición para especies fluviais. Ademais, o seu ciclo de vida, caracterizado por una forte dependencia dos peixes hóspedes, supón unha boa oportunidade para avanzar no coñecemento científico sobre as interaccións bióticas entre especies. Deste xeito, este proxecto de tese foi levado a cabo nas redes fluviais enmarcadas dentro dos límites administrativos de Galicia (noroeste de España) onde os peixes hóspedes para *M. margaritifera* son o salmón (*Salmo salar)* e a troita común (*Salmo trutta*), xunto co ecotipo migrador anádromo desta última, o reo. Trátase, así mesmo, da primeira tese realizada sobre esta especie na Península Ibérica, polo que nela se amosan os primeiros datos actualizados sobre a súa distribución, abundancia e estado de conservación neste territorio.

Así, o primeiro capítulo consiste nunha revisión xeral sobre os antecedentes e a necesidade deste traballo. No capítulo 2 preséntanse os primeiros datos actualizados sobre a distribución da especie en Galicia, así como unha estima da súa abundancia. Por outra banda, durante a mostraxe detectaouse a presenza doutras species (Lois *et al.*, 2009) como ameixa asiática invasora (*Corbicula fluminea)* (Anexo II). Para cubrir estes obxectivos foi deseñada unha mostraxe específica, na que se combinaron métodos cualitativos e cuantitativos dirixidos, non só para detectar a presenza da especie, senón tamén a existencia de recrutamento. A análise destes resultados foi clave para elaborar a diagnose do estado de conservación da náiade neste territorio.

Os resultados amosan que *M. margaritifera* se atopa en 54 ríos de Galicia, 27 dos cales non foran documentados antes, e aportan información sobre 522 novas localidades nos que a especie está presente e nas que se estimaron as súas abundancias. Galicia conta, polo tanto, co maior número de poboacións de *M. margaritifera* da Península Ibérica, observándose recrutamento aparente en 11 ríos (Lois *et al.*, 2014). Deste xeito, do sistema de mostraxe deseñado neste traballo resultou unha técnica efectiva para cubrir grandes áreas, así como para cando a especie presenta unha distribución marcadamente estratificada (Villella & Smith, 2005). O estudo e os resultados extraídos desta sección aportaron os datos básicos

necesarios para deseñar e desenvolver a modelaxe e a predición realizadas nos posteriores capítulos da tese.

O capítulo 3 céntrase no uso de modelos de distribución das especies, baseados nos datos actualizados da presenza da náiade na área de estudo. O algoritmo de entropía máxima (MaxEnt) (Phillips *et al.,* 2006; Phillips & Dudík, 2008), que utiliza só datos de presenza, foi utilizado para predición da presenza do mexillón de río e para avaliar o efecto das inclusión das interaccións bióticas na modelización. Neste contexto, seleccionouse un conxunto de 16 variables preditivas pertencentes a catro categorías (xeoloxía, clima, relevo e factores bióticos). Estes preditores foron elexidos para seren usados como covariables, con unha resolución espacial de 40x40 metros. As interaccións bióticas foron representadas nas predicións como abundancia e biomasa dos peixes hóspedes.

Os resultados deste estudo indican que os efectos das interaccións bióticas, entre *M. margaritifera* e os seus hóspedes, son perceptibles a escala rexional (todas as redes fluviais galegas), e discernibles nas predicións a escala local. O grado de axuste dos modelos foi testado mediante a área baixo a curva (AUC), que mostrou unha mellora significativa (un 4.5%) tras incluír as interaccións bióticas no modelo. Nesta mesma liña, as variables relacionadas cos peixes hóspedes contribuíron un 63% na predición da presenza do mexillón de ríos de Galicia. En relación ás variables, a biomasa de salmónidos e a precipitación media de verán foron factores que mais contribuíron no modelo (Lois *et al.*, 2015)

Os mapas de probabilidade de presenza permitiron detectar zonas específicas onde sería necesario aplicar un maior esforzo de investigación coa fin de cuantificar a calidade e estado actual do hábitat. Estes mapas son una ferramenta útil a escala espacial fina, xa que serven para analizar as causas polas que a especie está ausente en áreas nas que a predición indica, polo contrario, que ofrecen un hábitat axeitado para a súa presenza. Alén diso, as predicións identificaron tamén novos afluentes, e tramos de río, onde as probabilidades de atopar a náiade son altas. Estes resultados indican,

polo tanto, novas localidades ao longo da rede fluvial, nas que é necesario concentrar estudios de detección e análise no futuro.

Con respecto á metodoloxía utilizada observouse como o MaxEnt xestionou, de forma eficaz, a complexidade no modelo resultante e axudou a discernir o efecto das interaccións bióticas. Neste caso de estudo, o desenvolvemento larval e a dispersión da especie dependen dun peixe hóspede, polo que a interacción entre eles é necesaria para que as poboacións de mexillón persistan e colonicen novos hábitats da rede fluvial.

O capítulo 4 presenta un proceso de modelado da abundancia de *M. margaritifera,* tendo en conta, ademais das interaccións bióticas, as características específicas dos ecosistemas fluviais. Con este obxectivo, as técnicas de Path análise (Wright, 1934) e os modelos xeoestatísticos, deseñados especificamente para redes fluviais (Peterson & Ver Hoef, 2010), foron aplicados aos datos de abundancia da especie. Nesta análise, de igual xeito que fixo para a de presenza no capítulo 2, utilizouse o mesmo conxunto de 16 variables preditivas pertencentes a catro categorías (xeoloxía, clima, relevo e factores bióticos).

Novamente, as diferentes técnicas aplicadas subliñan a importancia de incluír as interaccións bióticas ao modelar a abundancia a escala rexional. O Path análise axudou a discernir as interaccións entre variables, así como a explorar as diferentes fontes de heteroxeneidade. Por outra banda, tras aplicar o modelo xeoestatístico mixto, as diferenzas na varianza indicaron a necesidade de ter en conta a autocorrelación espacial intrínseca das redes fluviais nos procesos de modelado. Estes modelos xeoespaciais lineais mixtos teñen en conta os procesos relacionados co fluxo direccional da auga, e, polo tanto, axudan a reter información relevante para a comprensión de procesos fluviais e das interaccións bióticas. Co uso destes modelos, a varianza explicada para a densidade de *M. margaritifera* foi dun 52%, sendo a biomasa de peixes hospedeiros e a densidade de troita os únicos preditores significativos, a escala rexional, explicando un 2,4% da varianza para toda a área de estudo. En

contraste, os efectos aleatorios ("*Tail-down*" e "*Tail-up*") representaron un 49.5%, resultando a contribución euclidea non significativa.

Debido a que *M. margaritifera* se trata dun organismo bentónico, con escasa mobilidade, a autocovarianza "*Tail-down*", relacionada cos movementos activos en contra do fluxo de auga, representa a relación entre as náiades e o seu hóspede.Este compoñente contribuíu de xeito importante á autocorrelación espacial da abundancia de mexillóns e estimouse que presenta un rango xeostatísico de 774 m. Deste xeito, este compoñente indicaría a dispersion do mexillón augas ariba durante a etapa larval parasita mediante os movementos do seu peixe hóspede. Tamén é importante destacar que, debido a que *M. margaritifera* é un organismo moi lonxevo, este resultado podería estar indicando condicións anteriores no tempo, onde a dispersión río arriba polo peixe parasitado sucedeu en tramos non conectados polo fluxo da corrente fluvial.

Por outra band, estimouse que a autocovarianza "*Tail-up*", relacionada cos movementos pasivos dos organismos na dirección do fluxo da auga, ten un rango xeoestatístico de 17480 m. Este compoñente da varianza podería incluír outros procesos de dispersión río abaixo de *M. margaritifera,* como os desprazamentos por arrastre dos mexillóns polo aumento da corrente, a dispersión natural dos gloquidios ou incluso o desprazamento nesa dirección dos peixes parasitados. Deste xeito, o compoñente "*Tail-up*" tamén incluiría o efecto das interaccións bióticas na abundancia de *M . margaritifera* na rexión de estudo (Lois *et al.*, 2015).

Alén diso, no proceso de análise da abundancia os datos foron divididos en subgrupos (presenza vs. ausencia de peixes migradores), resultando que a varianza explicada foi a mais alta (78%) no subgrupo no que os peixes migradores que coexistían coas poboacións de mexillón. Así, os resultados do modelo indican que a presenza de salmónidos migradores é un factor clave que contribúe positivamente á abundancia do mexillón de río. Do mesmo xeito demostran a influencia indirecta dos encoros como barreiras físicas que fragmentan os ríos, e impiden o acceso dos hóspedes migratorios ás poboacións de *M. margaritifera* reducindo así a súa

abundancia no territorio. Na mesma liña, os resultados indican a importancia dos peixes hóspedes migradores para as poboación galegas de mexillón. Isto podería ser explicado polo feito de que os grandes salmónidos migradores posúen un maior rendemento reprodutivo (Pope *et al.,* 1961; Beacham & Murray, 1993; Elliott, 1995; Fleming, 1996; Dickerson *et al*., 2002; Milner *et al.*, 2003; Cowley, 2008), e a que realizan recorridos de maiores distancias dentro da rede fluvial (Milner *et al.,* 2003), o que estaría relacionado coa influencia positiva na abundancia de *M. margaritifera* nestas zonas.

Por outra banda, os resultados das análises mostraron a influencia das interaccións bióticas, con unha resolución xeoespacial nun rango de aproximadamente 17 km. As accións de conservación poderían ser aplicadas nesta escala na que se produce a autocorrelación, posto que se trata dun valor indicativo da extensión á que se producen estas interaccións entre peixes hóspedes e mexillóns. Esta escala espacial de autocorrelación, tamén podería ser utilizada como unha escala bioxeográfica práctica para a avaliación, a planificación, a xestión do hábitat e a administración dos programas de conservación, así coma no deseño de estratexias de mostraxe (Lois *et al.*, 2015). Polo tanto, e a modo de resumo, os patróns espaciais debidos ás interaccións bióticas poden ocorrer a gran escala nun sistema parasito-hóspede. Os resultados tanto do capítulo 3 coma do capítulo 4 mostran que as interaccións bióticas inflúen na distribución e abundancia de *M. margaritifera* a nivel rexional.

Seguidamente, no capítulo 5 descríbese un proceso de modelización dirixido a identificar as áreas de conservación para *M. margaritifera* e os seus peixes hóspedes. Con este fin foron realizadas predicións de abundancia que permitiron distinguir zonas onde ocorren as interaccións bióticas. A metodoloxía empregada para definir as unidades estratéxicas de conservación e xestión combinou a técnica xeoestatística de kriging (Krige, 1966), que interpola técnicas baseadas no coñecemento previo da dependencia espacial dos datos (Fortin & Dale, 2005), adaptada para redes fluviais (Ver Hoef & Peterson, 2014) e a estima da estrutura demográfica da poboación a través da construción dos perfís de distribución de idades. A avaliación da estrutura

poboacional de *M. margaritifera* nas áreas nas que se detectaron altas densidades, foi unha ferramenta útil para detectar as poboacións de mexillóns que se están a reproducir actualmente e nas que aínda se produce a interacción entre as náiades e os peixes hóspedes.

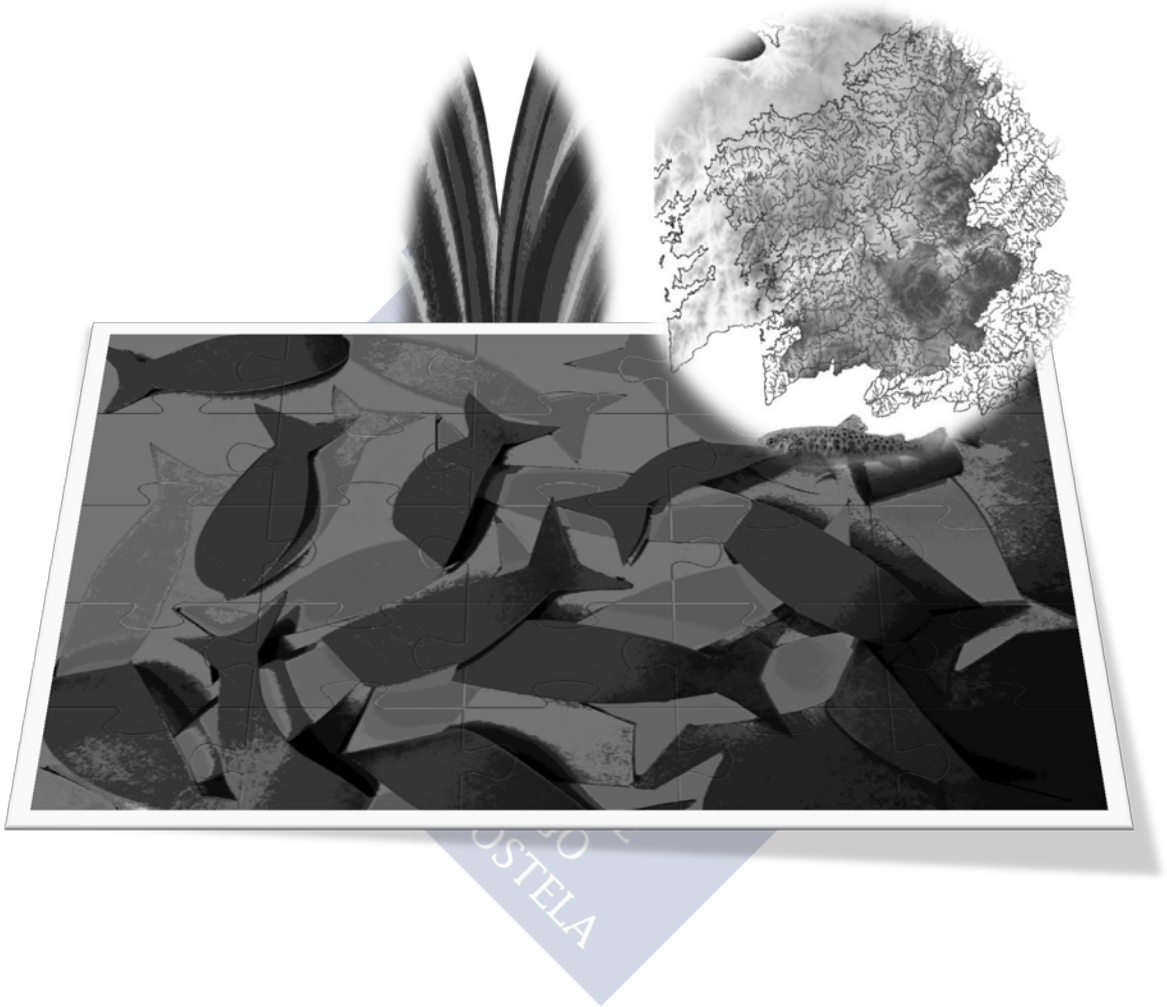
A combinación das predicións de abundancia, xunto cos perfís demográficos de idade das poboacións de náiades, analizadas xunto coas áreas de presenza dos peces migratorios hóspedes, foi esencial para identificar e clasificar as diferentes ou similares problemáticas, deseñar niveles de actuación e agrupar as diferentes zonas sobre as que definir estratexias de actuación comúns. Estas estratexias están claramente encamiñadas á preservación das interaccións entre os organismos implicados. As áreas onde as interaccións bióticas se seguen a producir, representadas por aquelas nas que polo menos un 20% da poboación posúe individuos de entre 5 a 10 anos, e nas que están presentes os peixes hóspedes migratorios, a protección debe estar enfocada sobre todo cara ás náiades, que terían que ser protexidas e monitorizadas. Neste caso atópanse os ríos Tea e Eo. No resto das zonas de conservación identificadas, terían que ser aplicadas simultaneamente estratexias enfocadas, tanto á protección de *M. margaritifera,* como dos seus peixes hóspedes.

Por outra banda, a existencia dunhas poucas poboacións de mexillón que presentan recrutamento poboacional augas arriba das presas, inaccesibles polo tanto aos salmónidos migratorios, como é caso do río Limia, suxiren a implantación de estratexias de xestión baseadas en garantir un número suficiente de peixes hóspedes dispoñibles para garantir o éxito do ciclo vital da náiade. Do mesmo xeito, a existencia de poboacións de mexillóns que carecen de recrutamento, en zonas onde están presentes os peixes migradores, podería ser indicativo de que a falla na renovación poboacional estea causada polos impactos antropoxénicos, como é o caso dos ríos Masma, Ouro, Mandeo e Landro. Para rematar, nas zonas onde non existe nin recrutamento poboacional nin peixes migradores suxírese a aplicación de medidas de xestión enfocadas á recuperación do hábitat e das poboacións de peixes hóspedes.

Por último, no capítulo 6 amósanse os resultados da primeira mostraxe piloto conducente a describir as características do microhábitat de *M. margaritifera* en Galicia. Esta avaliación inicial do microhábitat axudou a ter a o primeiro estudo piloto das características do microhábitat das poboacións da náiade en Galicia e servirá como base para o deseño de futuras estratexias de mostraxe.

Esta tese desenvolve un procedemento completo de mostraxe, modelización e predición para especies fluviais, centrándose especialmente nas interaccións bióticas entre especies. Así mesmo, demóstrase a necesidade de considerar a topoloxía direccional e a estrutura espacial do ecosistema fluvial na modelización e predición das especies fluviais. De forma específica, apórtase o primeiro estudo a gran escala sobre a relación de *M. margaritifera* e os seus hóspedes. Deste xeito, os coñecementos aportados por este traballo servirán como base para o deseño de estratexias de xestión para a conservación da especie tanto na Península Ibérica, coma en todo o seu rango de distribución, sendo tamén de aplicación a outras especies de náiades.

8. REFERENCES



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9. REFERENCES

Abràmoff M. D., P. J. Magalhães and S. J. Ram. 2004. Image processing with ImageJ. Biophotonics International **11**:36-43.

Akaike H. 1973. Information Theory and an Extension of the Maximum Likelihood Principle. Pages 267–281 *In* B.N. Petrov and F. Csaki, editors. Second International Symposium on Information Theory. Akademinai Kiado, Budapest.

Allan D., D. Erickson and J. Fay. 1997. The influence of catchment land use on stream integrity across multiple spatial scales. Freshwater Biology **37**:149-161.

Allan J.D, L. Johnson. 1997. Catchment-scale analysis of aquatic ecosystems. Freshwater Biology **37**:107-111.

Allan J. D. 2004. Landscapes and riverscapes: the influence of land use on stream ecosystems. Annual Review of Ecology, Evolution and Systematics **35**:257-284.

Álvarez-Claudio C., P. García-Rovés, R. Ocharan, J. Cabal, F. Ocharan and M. Álvarez. 2000. A new record of the freshwater pearl mussel *Margaritifera margaritifera* L. (Bivalvia, Unionoida) from the River Narcea (Asturias, north-western Spain). Aquatic Conservation: Marine and Freshwater Ecosystems **10**:93-102.

Araújo M. B., M. Luoto. 2007. The importance of biotic interactions for modelling species distributions under climate change. Global Ecology and Biogeography **16**:743-753.

Araújo M. B., A. T. Peterson. 2012. Uses and misuses of bioclimatic envelope modeling. Ecology **93**:1527-1539.

Araújo, M. B. and A. Rozenfeld. 2014. The geographic scaling of biotic interactions. Ecography **37**:406–415.

Araujo R. 2008. Mejillón de Rio *M. margaritifera* (L.). *In* J. R. Verdú and E. Galante, editors. Atlas de los Invertebrados Amenazados de España (especies en peligro crítico y en peligro). Organismo Autónomo Parques Nacionales.

Araujo R., M. Á. Ramos. 2001. Action plans for *Margaritifera auricularia* and *Margaritifera margaritifera* in Europe. Council of Europe.

Arvidsson B. L., J. Karlsson and M. E. Österling. 2012. Recruitment of the threatened mussel *Margaritifera margaritifera* in relation to mussel population size, mussel density and host density. Aquatic Conservation: Marine and Freshwater Ecosystems **22**:526-532.

Austin M. 2002. Spatial prediction of species distribution: an interface between ecological theory and statistical modelling. Ecological Modelling **157**:101-118.

Azpeitia F. 1933. Conchas bivalvas de agua dulce de España y Portugal. Memorias del Instituto Geológico y Minero de España.Madrid **1**:458.

Bauer G. 1986. The status of the freshwater pearl mussel *Margaritifera margaritifera* L. in the South of its European range. Biological Conservation **38**:1-9.

Bauer G. 1987. Reproductive strategy of the freshwater pearl mussel *Margaritifera margaritifera*. The Journal of Animal Ecology **56**:691-704.

Bauer G. 1988. Threats to the freshwater pearl mussel *Margaritifera margaritifera* L. in central Europe. Biological Conservation **45**:239-253.

Bauer G. 1991. Plasticity in Life History Traits of the Freshwater Pearl Mussel-Consequences for the Danger of Extinction and for Conservation Measures. Pages 103-120 *In* A. Seitz u. V. Loeschcke editors. Species conservation: A Population-Biological Approach. Birkhäuser Verlag.

Bauer G. 1992. Variation in the life span and size of the freshwater pearl mussel. Journal of Animal Ecology **61**: 425–436.

Bauer G. 2001. Life-history variation on different taxonomic levels of naiads. Pages 83-92 *In* Bauer G, Wächtler K editors. Ecology and evolution of the freshwater mussels Unionoida Springer, New York.

Beacham T., C. Murray. 1993. Fecundity and egg size variation in North American Pacific salmon (*Oncorhynchus*). Journal of fish biology **42**:485-508.

Beasley C., D. Roberts. 1996. The current distribution and status of the freshwater pearl mussel *Margaritifera margaritifera* L. 1758 in north-west Ireland. Aquatic Conservation: Marine and Freshwater Ecosystems **6**:169-177.

Beasley C., D. Roberts and T. Mackie. 1998. Does the freshwater pearl mussel, *Margaritifera margaritifera* L., face extinction in Northern Ireland?. Aquatic Conservation: Marine and Freshwater Ecosystems **8**:265-272.

Behnke, R. J. 2002. Trout and salmon of North America. Chantileer Press, Inc., New York, USA.

Beier U., E. Degerman, A. Melcher, C. Rogers and H. Wirlöf. 2007. Processes of collating a European fisheries database to meet the objectives of the European Union Water Framework Directive. Fisheries Management and Ecology **14**:407-416.

Bespalaya Y. V., I. N. Bolotov and A. A. Makhrov. 2007. State of the population of the European pearl mussel *Margaritifera margaritifera* (L.) (Mollusca, Margaritiferidae) at the northeastern boundary of its range (Solza River, White Sea Basin). Russian Journal of Ecology **8**:265-272.

Beyer H.2004. Hawth's analysis tools for ArcGIS. (http://www.spatialecology.com).

Bogan A. E. 1993. Freshwater bivalve extinctions (Mollusca: Unionoida): a search for causes. American Zoologist **33**:599-609.

Bogan A. E. 2008. Global diversity of freshwater mussels (Mollusca, Bivalvia) in freshwater. Hydrobiologia **595**:139-147.

Bond N., P. Lake. 2003. Local habitat restoration in streams: constraints on the effectiveness of restoration for stream biota. Ecological Management & Restoration **4**:193-198.

Boulangeat I., D. Gravel and W. Thuiller. 2012. Accounting for dispersal and biotic interactions to disentangle the drivers of species distributions and their abundances. Ecology Letters **15**:584-593.

Bouza C., J. Castro, P. Martinez, R. Amaro, C. Fernández, P. Ondina, A. Outeiro and E. San Miguel. 2007. Threatened freshwater pearl mussel *Margaritifera margaritifera* L. in NW Spain: low and very structured genetic variation in southern peripheral populations assessed using microsatellite markers. Conservation Genetics **8**:937-948.

Brown J. H. 1984. On the relationship between abundance and distribution of species. The American naturalist **2**:255-279.

Brown J. H., D. W. Mehlman and G. C. Stevens. 1995. Spatial variation in abundance. Ecology **76**:2028-2043.

Buddensiek V., H. Engel, S. Fleischauer-Rossing and K. Wachtler. 1993. Studies on the chemistry of interstitial water taken from defined horizons in the fine sediments of bivalve habitats in several northern German lowland waters. II: microhabitats of *Margaritifera margaritifera* L., *Unio crassus* (Philipsson) and *Unio tumidus* Philipsson. Archiv für Hydrobiologie **127**:151-166.

Burch J. B. 1973. Freshwater unionacean clams (Mollusca: Pelecypoda) of North America. **14**:12-894.

Caballero P., F. Cobo and M. A. González. 2006. Life history of a sea trout (*Salmo trutta* L.) population from the north-west Iberian Peninsula (River Ulla, Galicia, Spain). Pages 234-247 *In* Sea trout: biology, conservation and management, Proceedings of the First International Sea Trout Symposium, Cardiff, July 2004, Blackwell Publishing Ltd, Oxford, UK.

Cawley E. 1993. Sampling adequacy in population studies of freshwater mussels. Pages 14-14 *In* Cummings K.S., Buchanan A.C. and L.M Koch editors. Conservation and management of freshwater mussels. Proceedings of an Upper Mississippi River

Conservation Committee (UMRCC) Symposium, St. Louis, Missouri, October 1992, Upper Mississippi River Conservation Committee: Rock Island, Illinois.

Channell R., M. V. Lomolino. 2000a. Dynamic biogeography and conservation of endangered species. Nature **403**:84-86.

Channell R., M. V. Lomolino. 2000b. Trajectories to extinction: spatial dynamics of the contraction of geographical ranges. Journal of Biogeography **27**:169-179.

Chesson P. 2000. Mechanisms of maintenance of species diversity. Annual Review of Ecology and Systematics **31**:343-366.

Cosgrove P., M. Young, L. Hastie, M. Gaywood and P. Boon. 2000. The status of the freshwater pearl mussel *Margaritifera margaritifera* Linn. in Scotland. Aquatic Conservation: Marine and Freshwater Ecosystems **10**:197-208.

Cosgrove P., D. Donald, F. Cosgrove, E. Farquhar,D. Jarrett, S. Jancke, A. Mitchell and R. Moggach. 2014. Population size, structure and distribution of an unexploited freshwater pearl mussel *Margaritifera margaritifera* (L.) population in Scotland. Journal of Conchology **5**: 541-552.

Council of European Comunities. 1992. Directive 92/43/EEC of the Council of the European Communities of 21st May 1992 on the conservation of natural habitats and of wild fauna and flora. Official Journal of the European Communities **L206**:7-50.

Cowley D. E. 2008. Estimating required habitat size for fish conservation in streams. Aquatic Conservation: Marine and Freshwater Ecosystems **18**:418-431.

Cressie N., D. O'Donnell. 2010. Comment: Statistical dependence in stream networks. Journal of the American Statistical Association **105**: 18-21.

Cressie N., J. Frey, B. Harch and M. Smith. 2006. Spatial prediction on a river network. Journal of Agricultural, Biological, and Environmental Statistics **11**:127-150.

de Araújo C. B., L. O. Marcondes-Machado and G. C. Costa. 2014. The importance of biotic interactions in species distribution models: a test of the Eltonian noise hypothesis using parrots. Journal of Biogeography **41**: 513–523.

Dickerson B., T. Quinn and M. Willson. 2002. Body size, arrival date, and reproductive success of pink salmon, Oncorhynchus gorbuscha. Ethology Ecology & Evolution **14**:29-44.

Diniz-Filho J. A. F., L. M. Bini and B. A. Hawkins. 2003. Spatial autocorrelation and red herrings in geographical ecology. Global Ecology and Biogeography **12**:53-64.

Douda K., P. Horký and M. Bílý. 2012. Host limitation of the thick-shelled river mussel: identifying the threats to declining affiliate species. Animal Conservation **15**:536-544.

Dudgeon D., A. H. Arthington, M. O. Gessner, Z. Kawabata, D. J. Knowler, C. Lévêque, R. J. Naiman, A. Prieur-Richard, D. Soto and M. L. Stiassny. 2006. Freshwater biodiversity: importance, threats, status and conservation challenges. Biological reviews **81**:163-182.

Dunn R. R., N. C. Harris, R. K. Colwell, L. P. Koh and N. S. Sodhi. 2009. The sixth mass coextinction: are most endangered species parasites and mutualists?. Proceedings of the Royal Society B: Biological Sciences **276**:3037-3045.

Elith J., J. R. Leathwick. 2009. Species distribution models: ecological explanation and prediction across space and time. Annual Review of Ecology, Evolution, and Systematics **40**:677-697.

Elith J., S. J. Phillips, T. Hastie, M. Dudík, Y. E. Chee and C. J. Yates. 2011. A statistical explanation of MaxEnt for ecologists. Diversity and Distributions **17**:43-57.

Elith J., C. H. Graham, R. P. Anderson, M. Dudik, S. Ferrier, A. Guisan, R. J. Hijmans, F. Huettmann, J. R. Leathwick and A. Lehmann. 2006a. Novel methods improve prediction of species' distributions from occurrence data. Ecography **29**:129-151.

Elith J., C. H. Graham, R. P. Anderson, M. Dudík, S. Ferrier, A. Guisan, R. J. Hijmans, F. Huettmann, J. R. Leathwick, A. Lehmann, J. Li, L. G. Lohmann, B. A. Loiselle, G. Manion, C. Moritz, M. Nakamura, Y. Nakazawa, J. McC. M. Overton, A. Townsend Peterson, S. J. Phillips, K. Richardson, R. Scachetti-Pereira, R. E. Schapire, J. Soberón, S. Williams, M. S. Wisz and N. E. Zimmermann. 2006b. Novel methods improve prediction of species? distributions from occurrence data. Ecography **29**:129-151.

Elliott J. 1995. Fecundity and egg density in the redd for sea trout. Journal of fish biology **47**:893-901.

Elliott S. R., T. A. Coe, J. M. Helfield and R. J. Naiman. 1998. Spatial variation in environmental characteristics of Atlantic salmon (*Salmo salar*) rivers. Canadian Journal of Fisheries and Aquatic Sciences **55**:267-280.

ESRI. 2009. ArcGIS Desktop: Release 9.3. Environmental Systems Research Institute, Redlands, California, USA.

Fausch K. D., C. E. Torgersen, C. V. Baxter and H. W. Li. 2002. Landscapes to Riverscapes: Bridging the Gap between Research and Conservation of Stream Fishes. Bioscience **52**:483-498.

Fernández de la Cigoña E. 2004. A Voltas coa Fauna Galega (Traballos e Novas sobre Invertebrados, Peixes, Réptiles, Aves e Maníferos da Nosa Terra). Colección Natureza galega.Vol.XVIII. Asociación Galega para a Cultura e a Ecoloxía, Vigo, Pontevedra.

Fleming I. A. 1996. Reproductive strategies of Atlantic salmon: ecology and evolution. Reviews in Fish Biology and Fisheries **6**:379-416.

Fortin M., M. R. T. Dale. 2005. Spatial analysis: a guide for ecologists. Cambridge University Press, Cambridge, UK.

Fortin M. J., T. Keitt, B. Maurer, M. Taper, D. M. Kaufman and T. Blackburn. 2005. Species' geographic ranges and distributional limits: pattern analysis and statistical issues. Oikos **108**:7-17.

García de Leaniz C. G. 2008. Weir removal in salmonid streams: implications, challenges and practicalities. Hydrobiologia **609**:83-96.

Garcia de Leaniz, C. and J. J. Martinez. 1988. The Atlantic salmon in the rivers of Spain with particular reference to Cantabria. Pages 179-209 *In* Mills, D. and D. Piggins, editors. Atlantic salmon: planning for the future. The Proceedings of the Third International Atlantic Salmon Symposium – held in Biarritz, France, 21-23 October, 1986, Timber Press, Portland, OR, USA.

Garreta V., P. Monestiez and J. M. Ver Hoef. 2010. Spatial modelling and prediction on river networks: up model, down model or hybrid?. Environmetrics **21**:439-456.

Gaston K. J. 1990. Patterns in the geographical ranges of species. Biological Reviews **65**:105-129.

Geist J. 2010. Strategies for the conservation of endangered freshwater pearl mussels (*Margaritifera margaritifera* L.): a synthesis of Conservation Genetics and Ecology. Hydrobiologia **644**:69-88.

Geist J., K. Auerswald. 2007. Physicochemical stream bed characteristics and recruitment of the freshwater pearl mussel (*Margaritifera margaritifera*). Freshwater Biology **52**:2299-2316.

Geist J., M. Porkka and R. Kuehn. 2006. The status of host fish populations and fish species richness in European freshwater pearl mussel (*Margaritifera margaritifera*) streams. Aquatic Conservation: Marine and Freshwater Ecosystems **16**: 251–266

Gomi T., R. C. Sidle and J. S. Richardson. 2002. Understanding processes and downstream linkages of headwater systems. Bioscience **52**:905-916.

Graells M. P. 1870. Exploración científica de las costas del departamento marítimo de Ferrol verificada de orden del Almirantazgo por el vocal de la Comisión Permanente de Pesca D. Mariano de la Paz Graells en el verano de 1869, Madrid: Impr. Fortanet.

Graf D. L., K. S. Cummings. 2007. Review of the systematics and global diversity of freshwater mussel species (Bivalvia: Unionoida). Journal of Molluscan Studies **73**:291-314.

Graham C. H., J. Elith, R. J. Hijmans, A. Guisan, A. T. Peterson, B. A. Loiselle and The Nceas Predicting Species Distributions Working Group. 2008. The influence of spatial errors in species occurrence data used in distribution models. Journal of Applied Ecology **45**:239-247.

Grande C., R. Araujo and M. Ramos. 2001. The gonads of *Margaritifera auricularia* (Spengler, 1793) and *M. margaritifera* (Linnaeus, 1758) (Bivalvia: Unionoidea). Journal of Molluscan Studies **67**:27.

Gum B., M. Lange and J. Geist. 2011. A critical reflection on the success of rearing and culturing juvenile freshwater mussels with a focus on the endangered freshwater pearl mussel (*Margaritifera margaritifera* L.). Aquatic Conservation: Marine and Freshwater Ecosystems **21**:743-751.

Haag, W. R. and M. L. Warren, Jr. 1998. Role of ecological factors and reproductive strategies in structuring freshwater mussel communities. Role of ecological factors and reproductive strategies in structuring freshwater mussel communities. Canadian Journal of Fisheries and Aquatic Sciences **55**:297-306.

Hampe A., R. J. Petit. 2005. Conserving biodiversity under climate change: the rear edge matters. Ecology Letters **8**:461-467.

Harris G. P. 1998. Predictive models in spatially and temporally variable freshwater systems. Australian Journal of Ecology **23**:80-94.

Hastie L. C. 2011. Are Scottish freshwater pearl mussel populations recruiting normally?. Toxicological and Environmental Chemistry **93**: 1748-1763.

Hastie L. C., P.J. Cosgrove. 2001. The decline of migratory salmonid stocks: a new threat to pearl mussels in Scotland. Freshwater Forum **15**: 85–96.

Hastie L. C., P. J. Cosgrove. 2002. Intensive searching for mussels in a fast-flowing river: An estimation of sampling bias. Journal of Conchology **37**:309-316.

Hastie L. C., M. R. Young. 2003. Timing of spawning and glochidial release in Scottish freshwater pearl mussel (*Margaritifera margaritifera*) populations. Freshwater Biology **48**:2107-2117.

Hastie L. C., P. J. Boon and M. R. Young. 2000a. Physical microhabitat requirements of freshwater pearl mussels, *Margaritifera margaritifera* (L.). Hydrobiologia **429**:59-71.

Hastie L., P. Boon, M. Young and S. Way. 2001. The effects of a major flood on an endangered freshwater mussel population. Biological Conservation 98:107-115.

Hastie L, Tarr E, Al-Mousawi B, Young M. 2010. Medium-term recruitment patterns in Scottish freshwater pearl mussel *Margaritifera margaritifera* populations. Endangered Species Research **11**: 21-33.

Hastie L., M. Young, P. Boon, P. Cosgrove and B. Henninger. 2000b. Sizes, densities and age structures of Scottish *Margaritifera margaritifera* (L.) populations. Aquatic Conservation: Marine and Freshwater Ecosystems **10**:229-247.

Hauer F. R., M. S. Lorang. 2004. River regulation, decline of ecological resources, and potential for restoration in a semi-arid lands river in the western USA. Aquatic Sciences **66**:388-401.

Heikkinen R. K., M. Luoto, R. Virkkala, R. G. Pearson and J. Körber. 2007. Biotic interactions improve prediction of boreal bird distributions at macro-scales. Global Ecology and Biogeography **16**:754-763.

Hervella F., P. Caballero. 1999. Inventario piscícola dos ríos galegos. Xunta de Galicia, Santiago de Compostela, Spain.

Hervella F., P. Caballero. 2002. Situación actual del Salmón Atlantico en Galicia. Pages 27-37 *In* Garcia de Leaniz, C., Serdio, A., Consuerga, S., editors. El Salmón la joya de nuestros ríos, Gobierno de Cantabria.Consejeria de Ganadería y Pesca, Santander, Spain.

Hilborn R., S. C. Stearns. 1982. On inference in ecology and evolutionary biology: the problem of multiple causes. Acta Biotheoretica **31**:145-164.

Howard C., P. A. Stephens, J. W. Pearce-Higgins, R. D. Gregory and S. G. Willis. 2014. Improving species distribution models: the value of data on abundance. Methods in Ecology and Evolution **5**: 506-513.

Hugueny B. 1989. West African rivers as biogeographic islands: species richness of fish communities. Oecologia **79**:236-243.

Huston M. A. 1999. Local processes and regional patterns: appropriate scales for understanding variation in the diversity of plants and animals. Oikos **86** :393-401.

Hynes H. 1975. The stream and its valley. Edgardo Baldi memorial lecture. Verhandlungen der Internationalen Vereinigung fur theoretische und angewandte Limnologie **19**:1-15.

Isaak D. J., E. E. Peterson , J .M. Ver Hoef, S. J. Wenger, J. A. Falke., C. E. Torgersen, C. Sowder, S. E. Ashley, M. J. Fortin, C. E Jordan., A. S. Ruesch., N. Som and P. Monestiez. 2014. Applications of spatial statistical network models to stream data. WIREs Water **1**: 277-294

IUCN Red List of Threatened Species. 2013. http://www.iucnredlist.org/. [January 2013]

Johnson R. A., D. W. Wichern. 1982. Applied multivariate statistical analysis. Prentice-Hall, Englewood Cliffs, New Jersey, USA.

Jonsson B., N. Jonsson, E. Brodtkorb and P. Ingebrigtsen. 2001. Life-history traits of Brown Trout vary with the size of small streams. Functional Ecology **15**:310-317.

Kat P. W., G. M. Davis. 1984. Molecular genetics of peripheral populations of Nova Scotian Unionidae (Mollusca: Bivalvia). Biological Journal of the Linnean Society **22**:157-185.

Keitt T. H., O. N. Bjørnstad, P. M. Dixon and S. Citron-Pousty. 2002. Accounting for spatial pattern when modeling organism-environment interactions. Ecography **25**:616-625.

Kissling W. D., C. F. Dormann, J. Groeneveld, T. Hickler, I. Kühn, G. J. McInerny, J. M. Montoya, C. Römermann, K. Schiffers and F. M. Schurr. 2012. Towards novel approaches to modelling biotic interactions in multispecies assemblages at large spatial extents. Journal of Biogeography **39**:2163-2178.

Koh L. P., R. R. Dunn, N. S. Sodhi, R. K. Colwell, H. C. Proctor and V. S. Smith. 2004. Species coextinctions and the biodiversity crisis. Science **305**:1632-1634.

Krebs C J. 1999. Ecological Methodology. 2nd ed. Addison-Wesley Educational Publishers Inc.; Menlo Park, California.

Krige D. 1966. Two-dimensional weighted moving average trend surfaces for ore-evaluation. Journal of The South African Institute of Mining and Metallurgy **66**:13-38.

Legendre P. 1993. Spatial autocorrelation: trouble or new paradigm?. Ecology **74**:1659-1673.

Levin S. A. 1992. The problem of pattern and scale in ecology: the Robert H. MacArthur award lecture. Ecology **73**:1943-1967.

Lindenmayer D., M. Hunter. 2010. Some guiding concepts for conservation biology. Conservation Biology **24**:1459-1468.

Lois S. 2010. New records of *Corbicula fluminea* (Müller, 1774) in Galicia (Northwest of the Iberian Peninsula): Mero, Sil and Deva rivers. Aquatic Invasions **5**: S17–S20.

Lois S., P. Ondina, A. Outeiro, R. Amaro and E. San Miguel. 2014. The north-west of the Iberian Peninsula is crucial for conservation of *Margaritifera margaritifera* (L.) in Europe. Aquatic Conservation: Marine and Freshwater Ecosystems **24**:35-47.

Lois S., D.E Cowley, A. Outeiro, E. San Miguel, R. Amaro and P. Ondina. 2015. Spatial extent of biotic interactions affects species distribution and abundance in river networks: the freshwater pearl mussel and its hosts. Journal of Biogeography **42**: 229–240.

Lois S., R. Romero, R. Mascato, F. Rodríguez-Brea, E. San Miguel, A. Outeiro, R. Amaro and P. Ondina. 2009. As náides da bacía do Miño en Galiza. *In* IV Simpósio Ibérico sobre a bacía hidrografica do rio Minho (ed) C. Antunes, 140-143.

Loureiro J., G. Matía. 2001. El relieve. Pages 81-135 *In* Xunta de Galicia editor. Atlas de Galicia. Medio Natural, Xunta de Galicia, Santiago de Compostela.

Lynch M., B. Walsh. 2000. Genetics and analysis of quantitative traits. Sinauer Associates, Inc., Sunderland, MA, USA.

Macho J. 1878. Moluscos de auga dulce de Galicia, con observaciones sobre las especies y localidades **7**:131-190.

Machordom A., R. Araujo, D. Erpenbeck and M. Á. Ramos. 2003. Phylogeography and conservation genetics of endangered European Margaritiferidae (Bivalvia: Unionoidea). Biological Journal of the Linnean Society **78**:235-252.

Marco-Rius F., P. Caballero, P. Morán and C. García de Leaniz. 2012. And the Last Shall Be First: Heterochrony and Compensatory Marine Growth in Sea Trout (*Salmo trutta*). PLoS ONE **7** (10):e45528.

Matheron G. 1963. Principles of geostatistics. Economic geology **58**:1246-1266.

Meier E. S., F. Kienast, P. B. Pearman, J. Svenning, W. Thuiller, M. B. Araújo, A. Guisan and N. E. Zimmermann. 2010. Biotic and abiotic variables show little redundancy in explaining tree species distributions. Ecography **33**:1038-1048.

Meyers T. R., R. E. Millemann. 1977. Glochidiosis of salmonid fishes. I. Comparative susceptibility to experimental infection with *Margaritifera margaritifera* (L.) (Pelecypoda: Margaritanidae). The Journal of parasitology **63**:728-733.

Milner N. J., J. M. Elliott, J. D. Armstrong, R. Gardiner, J. S. Welton and M. Ladle. 2003. The natural control of salmon and trout populations in streams. Fisheries Research **62**:111-125.

Minshall G. W., D. A. Andrews and C. Y. Manuel-Faler. 1983. Application of island biogeographic theory to streams: macroinvertebrate recolonization of the Teton River, Idaho. Pages 279- 297 In J. R. Barnes and G. W. Minshall editors. Stream

ecology: application and testing of general ecological theory. Plenum Press, New York Stream Ecology, Springer.

Montalvo A. M., S. L. Williams, K. J. Rice, S. L. Buchmann, C. Cory, S. N. Handel, G. P. Nabhan, R. Primack and R. H. Robichaux. 1997. Restoration biology: a population biology perspective. Restoration Ecology **5**:277-290.

Montgomery D. R. 1999. Process domains and the river continuum. Journal of the American Water Resources Association **35**:397-410.

Moorkens E., I. Killeen and E. Ross. 2007. *Margaritifera margaritifera* (the freshwater pearl mussel) conservation assessment. Backing Document. A report to the National Parks and Wildlife Service, Dublin.

Morales J., A. Negro, M. Lizana, A. Martinez and J. Palacios. 2004. Preliminary study of the endangered populations of pearl mussel *Margaritifera margaritifera* (L.) in the River Tera (north-west Spain): habitat analysis and management considerations. Aquatic Conservation: Marine and Freshwater Ecosystems **14**:587-596.

Morisita M. 1959. Measuring of the dispersion and analysis of distribution patterns. Memoires of the Faculty of Science, Series E. Biology, Kyushu University **2**: 215–235.

Mutvei H., T. Westermark. 2001. How environmental information can be obtained from Naiad shells. Pages 367-379 *In* G. Bauer and K. Wachtler, editors. Ecology and Evolution of the Freshwater Mussels Unionoida, Ecological Studies No. 145, Springer Verlag, Heidelberg, Germany.

Nanson G. C., J. C. Croke. 1992. A genetic classification of floodplains. Geomorphology **4**:459-486.

Ninyerola, M., X. Pons and J.M. Roure. 2005. Atlas climático digital de la Península Ibérica: metodología y aplicaciones en bioclimatología y geobotanica. Universidad Autónoma de Barcelona, Bellaterra, Spain.

Olden J. D., D. A. Jackson. 2002. Illuminating the “black box”: a randomization approach for understanding variable contributions in artificial neural networks. Ecological Modelling **154**:135-150.

Oliver, G. 2000. Conservation objectives for the freshwater pearl mussel *(Margaritifera margaritifera*). Report to English Nature, Peterborough.

Ondina P., R. Romero, S. Lois, A. Outeiro, R. Amaro and E. San Miguel. 2010. Plan de Recuperación de *M. margaritifera* (L.). Xunta de Galicia.

Ondina P., C. Fernández, M. Hermida, R. Amaro, A. Outeiro and E. San Miguel. 2003. Datos preliminares sobre la presencia de *Margaritifera margaritifera* (Linnaeus,

1758) en los rios de Galicia. Pages 26-27 *In* XV Bienal de la Real Sociedad de Historia Natural: A Coruña, Spain.

Österling M. E., B. L. Arvidsson and L. A. Greenberg. 2010. Habitat degradation and the decline of the threatened mussel *Margaritifera margaritifera*: influence of turbidity and sedimentation on the mussel and its host. Journal of Applied Ecology **47**:759-768.

Ostrovsky A., I. Y. Popov. 2011. Rediscovery of the largest population of the freshwater pearl mussel (*Margaritifera margaritifera*) in the Leningrad oblast (north-west Russia). Aquatic Conservation: Marine and Freshwater Ecosystems **21**:113-121.

Outeiro A., P. Ondina, C. Fernámdez, R. Amaro and E. San Miguel. 2008. Population density and age structure of the freshwater pearl mussel, *Margaritifera margaritifera*, in two Iberian rivers. Freshwater Biology **53**:485-496.

Parrish D. L., R. J. Behnke, S. R. Gephard, S. D. McCormick and G. H. Reeves. 1998. Why aren't there more Atlantic salmon (*Salmo salar*)?. Canadian Journal of Fisheries and Aquatic Sciences **55**:281-287.

Pearson R. G., T. P. Dawson. 2003. Predicting the impacts of climate change on the distribution of species: are bioclimate envelope models useful?. Global Ecology and Biogeography **12**:361-371.

Pearson R. G., C. J. Raxworthy, M. Nakamura and A. T. Peterson. 2007. Predicting species distributions from small numbers of occurrence records: a test case using cryptic geckos in Madagascar. Journal of Biogeography **34**:102-117.

Peterson E. E., J. M. V. Hoef. 2010. A mixed-model moving-average approach to geostatistical modeling in stream networks. Ecology **91**:644-651.

Peterson E. E., J. M. Ver Hoef. 2014. STARS: An ArcGIS Toolset Used to Calculate the Spatial Information Needed to Fit Spatial Statistical Models to Stream Network Data. Journal of Statistical Software **56**:1-17.

Peterson E. E., J. M. Ver Hoef, D. J. Isaak, J. A. Falke, M. Fortin, C. E. Jordan, K. McNyset, P. Monestiez, A. S. Ruesch and A. Sengupta. 2013. Modelling dendritic ecological networks in space: an integrated network perspective. Ecology Letters **16**:707-719.

Phillips S. J., M. Dudík. 2008. Modeling of species distributions with Maxent: new extensions and a comprehensive evaluation. Ecography **31**:161-175.

Phillips S. J., R. P. Anderson and R. E. Schapire. 2006. Maximum entropy modeling of species geographic distributions. Ecological Modelling **190**:231-259.

Poff N. L. 1997. Landscape filters and species traits: towards mechanistic understanding and prediction in stream ecology. Journal of the North American Benthological Society :391-409.

Poff N. L., J. Ward. 1990. Physical habitat template of lotic systems: recovery in the context of historical pattern of spatiotemporal heterogeneity. Environmental management **14**:629-645.

Pope, J. A., D. H. Mills and W. M. Shearer. 1961. The fecundity of Atlantic salmon. The fecundity of Atlantic salmon.Freshwater Salmonid Fisheries Research, Scotland **26**:1-12.

Prié V., Q. Molina and B. Gamboa. 2014. French naiad (Bivalvia: Margaritiferidae, Unionidae) species distribution models: prediction maps as tools for conservation. Hydrobiologia **735**:81-94.

Quinlan E., C. Gibbins, I. Malcolm, R. Batalla, D. Vericat and L. Hastie. 2014. A review of the physical habitat requirements and research priorities needed to underpin conservation of the endangered freshwater pearl mussel *Margaritifera margaritifera.* Aquatic Conservation: Marine and Freshwater Ecosystems: doi: 10.1002/aqc.2484.

R Development Core Team. 2010. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Available at: http://www.r-project.org/.

Reis J. 2003.The freshwater pearl mussel [*Margaritifera margaritifera* (L.)] (Bivalvia, Unionoida) rediscovered in Portugal and threats to its survival. Biological Conservation **114**:447-452.

Rodríguez F. 2001. Os ríos galegos. Morfoloxía e réxime. Consello. Consello da Cultura Galega. Ponencia de Patrimonio Natural, Santiago de Compostela.

Rolán E., J. Otero-Schmitt. 1996. Guía dos moluscos de Galicia. Galaxia, Santiago de Compostela

Roth N. E., J. D. Allan and D. L. Erickson. 1996. Landscape influences on stream biotic integrity assessed at multiple spatial scales. Landscape Ecology **11**:141-156.

Rudzīte M. 2005. Assessment of the condition of freshwater pearl mussel *Margaritifera margaritifera* (Linnaeus 1758) populations in Latvia. Acta Universitatis Latviensis **691**:121-128.

San Miguel E. 1999. *Margaritifera margaritifera* en los rios de Galicia. *In* C. Nores, editor. Informe sobre la situación del Desmán Ibérico (Galemys pyrenaicus) en Espana. Seminario sobre Conservaicón de *Margaritifera margaritifera* y *Galemys*

pyrenaicus en la Penínusla iberica. Pola de Somiedo, Asturias (España). INDUROT. Universidad de Oviedo. Ministerio de Medio Ambiente, Spain.

San Miguel E., S. Monserrat, C. Fernandez, R. Amaro, M. Hermida, P. Ondina and C. Altaba. 2004. Growth models and longevity of freshwater pearl mussels (*Margaritifera margaritifera*) in Spain. Canadian journal of zoology **82**:1370-1379.

SAS Institute Inc. 2009. SAS/IML 9.22 User's Guide. SAS Institute, Inc, Cary NC,USA.

Schlosser I. J. 1991. Stream fish ecology: a landscape perspective. Bioscience **41**:704-712.

Schöne B. R., E. Dunca, J. Fiebig and M. Pfeiffer. 2005. Mutvei's solution: An ideal agent for resolving microgrowth structures of biogenic carbonates. Palaeogeography, Palaeoclimatology, Palaeoecology **228**:149-166.

Schwalb A. N., K. Cottenie, M. S. Poos and J. D. Ackerman. 2011. Dispersal limitation of unionid mussels and implications for their conservation. Freshwater Biology **56**:1509-1518.

Schweiger O., J. Settele, O. Kudrna, S. Klotz and I. Kühn. 2008. Climate change can cause spatial mismatch of trophically interacting species. Ecology **89**:3472-3479.

Seoane V. L. 1866. Reseña de la historia natural de Galicia.Edicións do castro, Seminario de Estudos Galegos, A Coruña, Spain.

Sepkoski J. J., M. A. Rex. 1974. Distribution of freshwater mussels: coastal rivers as biogeographic islands. Systematic Biology **23**:165-188.

Simberloff D. S. 1974. Equilibrium theory of island biogeography and ecology. Annual Review of Ecology and Systematics **5**:161-182.

Smith D. R. 2006. Survey design for detecting rare freshwater mussels. Journal of the North American Benthological Society **25**:701-711.

Snyder N. F. R., S. R. Derrickson, S. R. Beissinger, J. W. Wiley, T. B. Smith, W. D. Toone and B. Miller. 1996. Limitations of captive breeding in endangered species recovery. Conservation Biology **10**:338-348.

Soberon J., M. Nakamura. 2009. Niches and distributional areas: concepts, methods and assumptions. Proceedings of the National Academy of Sciences of the United States of America **106**:19644-19650.

Som N.A., Monestiez P., Ver Hoef J.M., Zimmerman, D.L. and Peterson, E.E.2014. Spatial sampling on streams: principles for inference on aquatic networks. Environmetrics **25**: 306–323.

Soulé M. E., J. A. Estes, B. Miller and D. L. Honnold. 2005. Strongly interacting species: conservation policy, management, and ethics. Bioscience **55**:168-176.

Sousa R., Â. Amorim, E. Froufe, S. Varandas, A. Teixeira and M. Lopes-Lima. 2014. Conservation status of the freshwater pearl mussel *Margaritifera margaritifera* in Portugal. Limnologica - Ecology and Management of Inland Waters doi:10.1016/j.limno.2014.07.004

Spooner D. E., C. C. Vaughn. 2008. A trait-based approach to species' roles in stream ecosystems: climate change, community structure, and material cycling. Oecologia **158**:307-317.

Stanford J. A., J. Ward. 1993. An ecosystem perspective of alluvial rivers: connectivity and the hyporheic corridor. Journal of the North American Benthological Society **12** :48-60.

Sutherland W.J., Pullin A.S., Dolman P.M., Knight, T.M. 2004. The need for evidence-based conservation. Trends in Ecology and Evolution. **19**: 305-308.

Stork N. E., C. H. Lyal. 1993. Extinction or 'co-extinction' rates?. Nature **366**:307.

Strahler A. N. 1957. Quantitative analysis of watershed geomorphology.Transactions of the American Geophysical Union 38:913-920. **38**:913-920.

Strayer DL. 2008. Freshwater mussel ecology: a multifactor approach to distribution and abundance. University of California Press: Berkeley, California.

Strayer D. L., D. R. Smith. 2003. A guide to sampling freshwater mussel population. American Fisheries Society (ed) American Fisheries Society Monograph 8: Bethesda, Maryland.

Strayer D. L., J. A. Downing, W. R. Haag, T. L. King, J. B. Layzer, T. J. Newton and S. J. Nichols. 2004. Changing perspectives on pearly mussels, North America's most imperiled animals. Bioscience **54**:429-439.

Syfert M. M., M. J. Smith and D. A. Coomes. 2013. The effects of sampling bias and model complexity on the predictive performance of MaxEnt species distribution models. PloS ONE **8**:e55158.

Taeubert J., M. Denic, B. Gum, M. Lange and J. Geist. 2010. Suitability of different salmonid strains as hosts for the endangered freshwater pearl mussel (*Margaritifera margaritifera* L.). Aquatic Conservation: Marine and Freshwater Ecosystems **20**:728-734.

Terui A., Y. Miyazaki, A. Yoshioka, K. Kaifu, S. S. Matsuzaki and I. Washitani. 2014. Asymmetric dispersal structures a riverine metapopulation of the freshwater pearl mussel *Margaritifera laevis*. Ecology and Evolution **4**:3004-3014.

Teixeira A, Lopes-Lima M, Machado J, Hinzmann M, Cortes RMV, Varandas S, Antunes F. 2010. Estudos preliminares de populações de mexilhão-de-rio (*Margaritifera margaritifera* L.) nos rios Rabaçal e Tuela (Nordeste de Portugal): análise do habitat e da qualidade da água e sedimentos. 10º Congresso da água, Alvor. Portugal

Townsend C. R. 1996. Concepts in river ecology: pattern and process in the catchment hierarchy. Archiv für Hydrobiologie, Supplement **113**:3-21.

Valovirta I. 1995. Jokihelmisimpukkaa tutkitaan ja suojellaan. (Summary: Research and conservation of freshwater pearl mussel). – Luonnontieteelinen keskusmuseo

Vannote R. L., G. W. Minshall, K. W. Cummins, J. R. Sedell and C. E. Cushing. 1980. The river continuum concept. Canadian Journal of Fisheries and Aquatic Sciences **37**:130-137.

Varandas S., M. Lopes-Lima., A. Teixeira, M. Hinzmann, J. Reis,R. Cortes., J. Machado and R. Sousa. 2013. Ecology of southern European pearl mussels (*Margaritifera margaritifera*): first record of two new populations on the rivers Terva and Beça (Portugal). Aquatic Conservation: Marine and Freshwater Ecosystems **23**: 374–389.

Vaughn C. C. 1997.Regional patterns of mussel species distributions in North American rivers. Ecography **20**:107-115.

Vaughn C, Taylor, C. 1999. Impoundments and the decline of freshwater mussels: a case study of an extinction gradient. Conservation Biology **13**: 912–920.

Vaughn C. C., C. M. Taylor. 2000. Macroecology of a host-parasite relationship. Ecography **23**:11-20.

Vaughn C., Taylor C.M., Eberhard K.J. 1997. A comparison of the effectiveness of timed searches vs. quadrat sampling in mussel surveys. Pages 157-162 *In* K.S. Cummings, A.C. Buchanan, C.A. Mayer and T.J. Naimo editors. Conservation and Management of Freshwater Mussels II: Initiatives for the Future. Upper Mississippi Conservation Commitee, Rock Island, Illinois.

Vaughn C. C., S. J. Nichols and D. E. Spooner. 2008. Community and foodweb ecology of freshwater mussels. Journal of the North American Benthological Society **27**:409-423.

Ver Hoef J. M., E. E. Peterson. 2010. A moving average approach for spatial statistical models of stream networks. Journal of the American Statistical Association **105**: 6-8.

Ver Hoef J. M., E. Peterson and D. Theobald. 2006. Spatial statistical models that use flow and stream distance. Environmental and Ecological Statistics **13**:449-464.

Ver Hoef J. M., E. E. Peterson, D. Clifford and R. Shah. 2014. SSN: an R package for spatial statistical modeling on stream networks. Journal of Statistical Software **56**:1-45.

Villella R. F., D. R. Smith. 2005. Two-phase sampling to estimate river-wide populations of freshwater mussels. Journal of the North American Benthological Society **24**:357-368.

Ward J. 1998. Riverine landscapes: biodiversity patterns, disturbance regimes, and aquatic conservation. Biological Conservation **83**:269-278.

Ward J., J. Stanford. 1995a. The serial discontinuity concept: extending the model to floodplain rivers. Regulated Rivers: Research & Management **10**:159-168.

Ward J. V., J. A. Stanford. 1995b. Ecological connectivity in alluvial river ecosystems and its disruption by flow regulation. Regulated Rivers: Research & Management **11**:105-119.

Ward J., K. Tockner, D. Arscott and C. Claret. 2002. Riverine landscape diversity. Freshwater Biology **47**:517-539.

Warren D. L., S. N. Seifert. 2011. Ecological niche modeling in Maxent: the importance of model complexity and the performance of model selection criteria. Ecological Applications **21**:335-342.

Watters G. T. 1996. Small dams as barriers to freshwater mussels (Bivalvia, Unionoida) and their hosts. Biological Conservation **75**:79-85.

Wiens J. A. 2002. Riverine landscapes: taking landscape ecology into the water. Freshwater Biology **47**:501-515.

Wiens J. J. 2011. The niche, biogeography and species interactions. Philosophical Transactions of the Royal Society of London **366**:2336-2350.

Williams J. D., M. L. Warren Jr, K. S. Cummings, J. L. Harris and R. J. Neves. 1993. Conservation status of freshwater mussels of the United States and Canada. Fisheries **18**:6-22.

Wilson C. D., D. Roberts. 2011a. Modelling distributional trends to inform conservation strategies for an endangered species. Diversity and Distributions 17:182-189.

Wilson C. D., D. Roberts and N. Reid. 2011b. Applying species distribution modelling to identify areas of high conservation value for endangered species: A case study using *Margaritifera margaritifera* (L.). Biological Conservation 144:821-829.

Wissmar R. C. 2004. Riparian corridors of eastern Oregon and Washington: Functions and sustainability along lowland-arid to mountain gradients. Aquatic Sciences-Research Across Boundaries **66**:373-387.

Wisz M. S., J. Pottier, W. D. Kissling, L. Pellissier, J. Lenoir, C. F. Damgaard, C. F. Dormann, M. C. Forchhammer, J. Grytnes and A. Guisan. 2013. The role of biotic interactions in shaping distributions and realised assemblages of species: implications for species distribution modelling. Biological Reviews **88**:15-30.

World Commission on Dams. 2000. Dams and Development: a new framework for decision-making: the Report of the World Commission on Dams. Earthscan Publications Ltd, London, UK and Sterling, VA, USA.

Wright S. 1934. The method of path coefficients. Annals of Mathematical Statistics **5**:161-215.

Young M., J. Williams. 1984. The reproductive biology of the freshwater pearl mussel *Margaritifera margaritifera* (Linn.) in Scotland. I: Field studies. Archiv für Hydrobiologie **99**:405-422.

Young M. R., P. J. Cosgrove and L. C. Hastie. 2001a. The extent of, and causes for, the decline of a highly threatened naiad: *Margaritifera margaritifera*. *In* Bauer G, Wächtler K editors. Ecology and evolution of the freshwater mussels Unionoida Springer, New York.

Young M.R., P.J. Cosgrove, L.C. Hastie and B. Henniger. 2001b. A Standardised method for assessing the status of freshwater mussels in clear, shallow rivers. Journal of Molluscan Studies **67**: 396–397.

Ziuganov V. A. Zotin, L. Nezlin and V. Tretiakov. 1994. The freshwater pearl mussels and their relationships with salmonid fish. VNIRO Publishing House.

Ziuganov V., E. San Miguel, R. J. Neves, A. Longa, C. Fernández, R. Amaro, V. Beletsky, E. Popkovitch, S. Kaliuzhin and T. Johnson. 2000. Life span variation of the freshwater pearl shell: a model species for testing longevity mechanisms in animals. AMBIO: A Journal of the Human Environment **29**:102-105.

9. ANNEXES

”Imola andando que a festa vai boa”

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ANNEX I

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A

B

Figure I.1.- Photos of river Miño (Ínsua de Seivane, Lugo); (A) winter conditions with high flow and (B) summer conditions with low flow. Photos by S. Lois.

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ANNEX II

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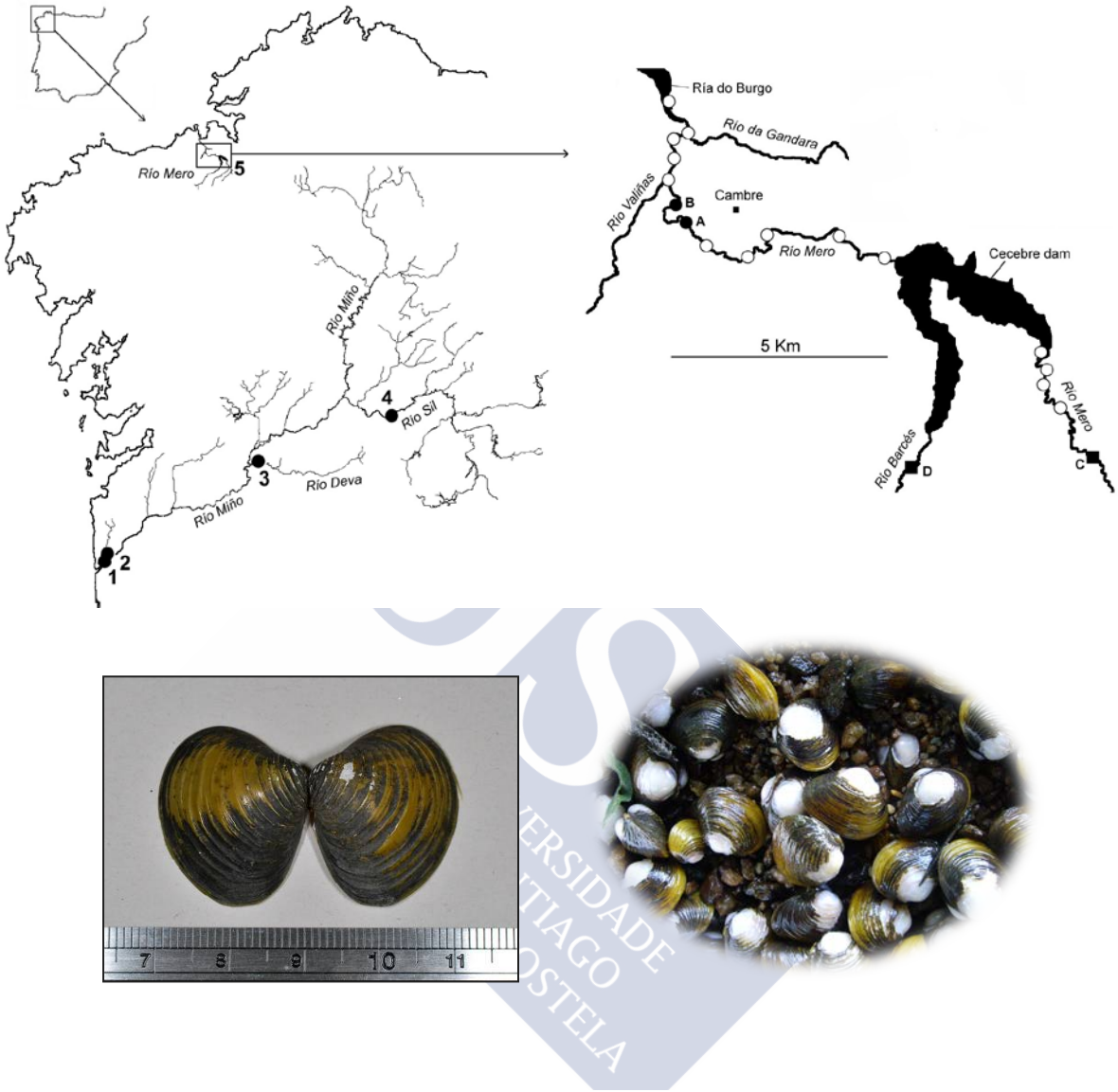

Figure II.1.- Distribution of *Corbicula fluminea,* Miño and Mero basins (Lois, 2010).

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ANNEX III

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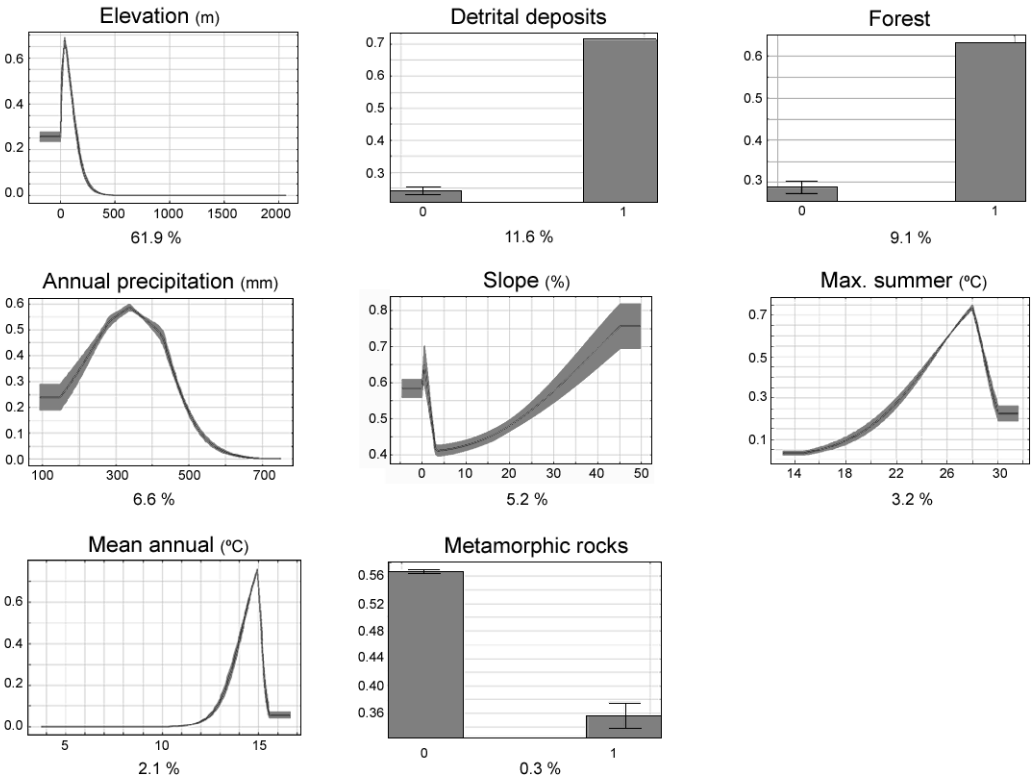
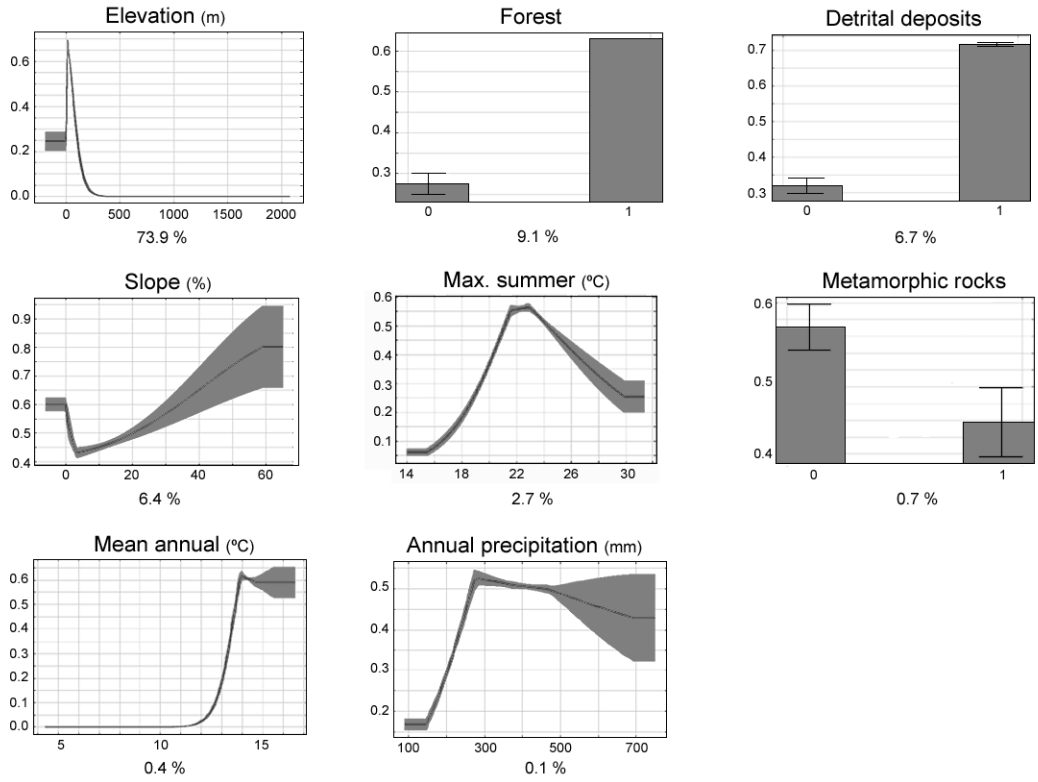
(a)


Elevation (m)
61.9 %
Detrital deposits
11.6 %
Forest
9.1 %
Annual precipitation (mm)
6.6 %
Slope (%)
5.2 %
Max. summer (ºC)
3.2 %
Mean annual (ºC)
2.1 %
Metamorphic rocks
0.3 %

(b)


Elevation (m)
73.9 %
Forest
9.1 %
Detrital deposits
6.7 %
Slope (%)
6.4 %
Max. summer (ºC)
2.7 %
Metamorphic rocks
0.7 %
Mean annual (ºC)
0.4 %
Annual precipitation (mm)
0.1 %

(c)

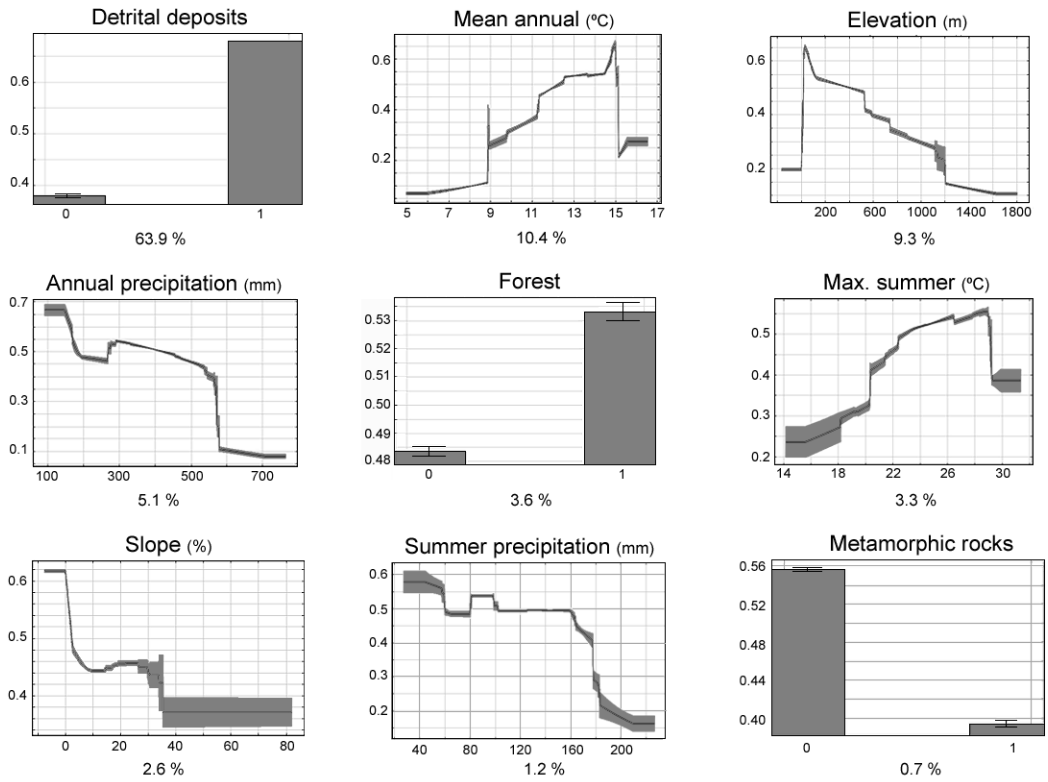


Figure III.1.- Partial dependence plots of variables in MaxEnt model of (a) Atlantic salmon, (b) migratory trout and (c) resident trout for predicting distribution of each host fish in the entire study area. The percent contribution of each variable in the MaxEnt model is shown below each graph; the y-axis in each graph indicates probability of host fish presence (logistic output) as a function of the variable indicated.

ANNEX IV

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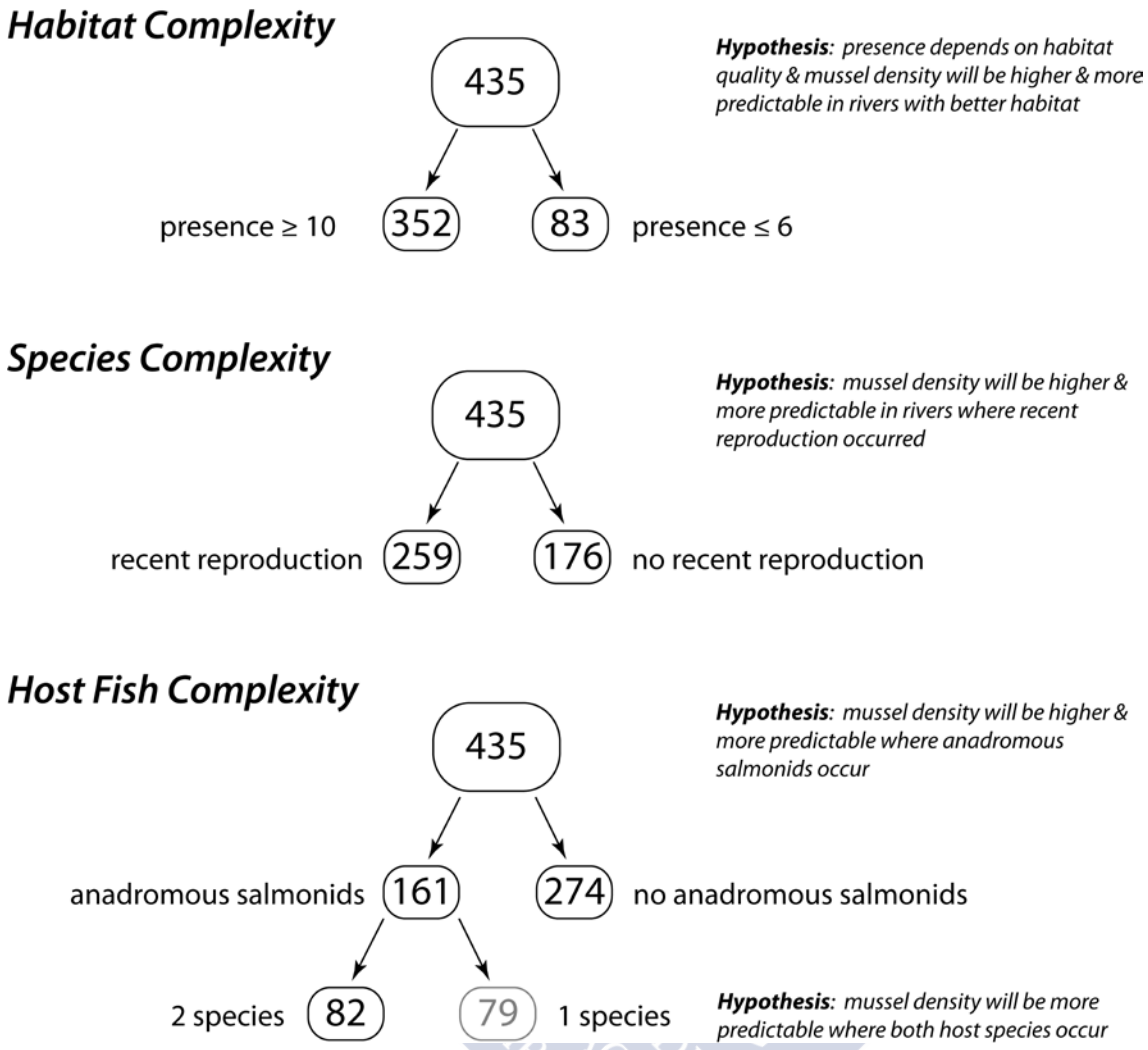

Figure IV.1.- Stratification of the data into various domains was used to explore possible sources of complexity associated with habitat, the parasitic mussel species and its hosts fish. The subset in which only 1 anadromous host fish was present could not be analised because the correlation matrix was singular.

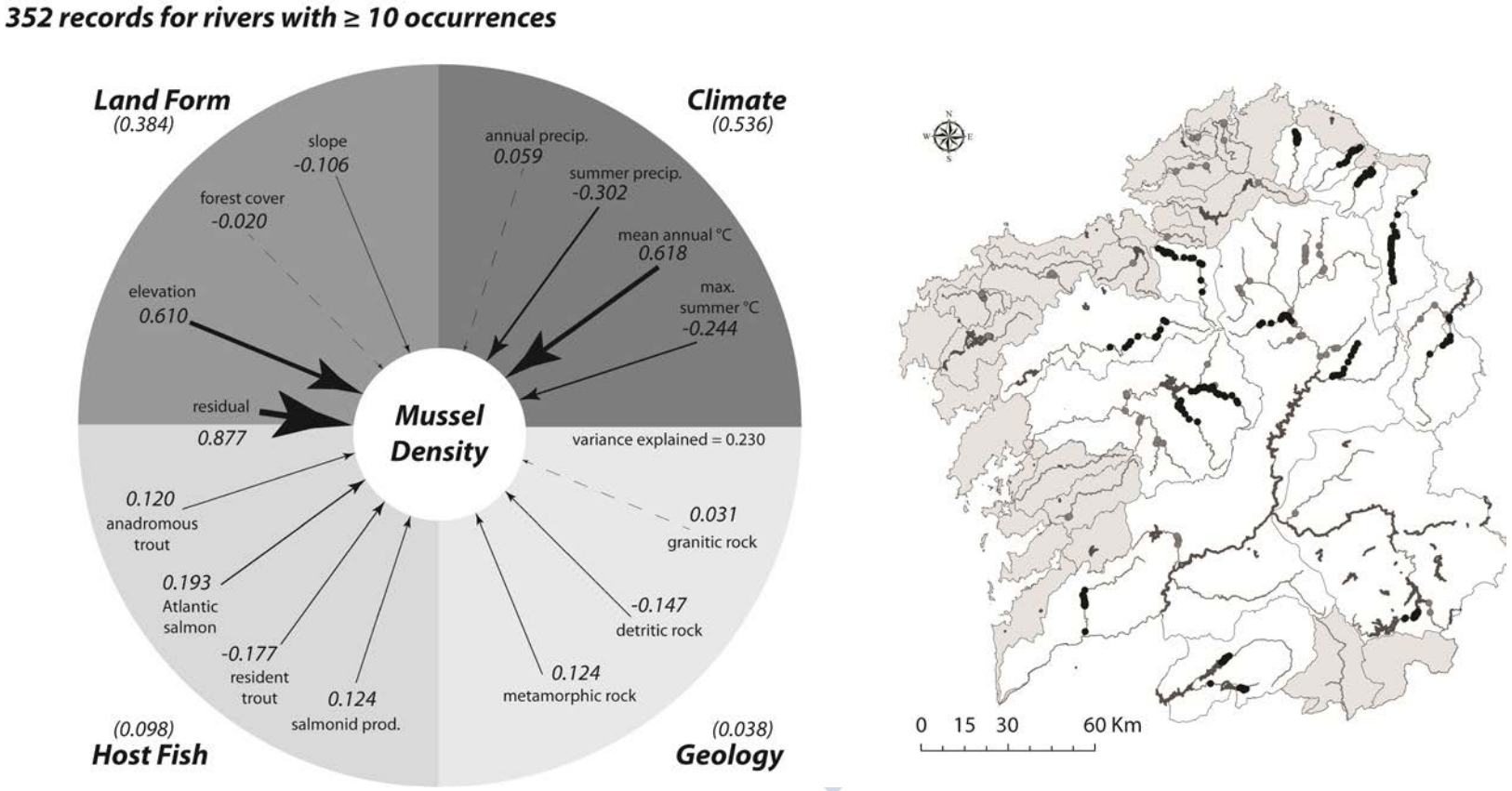

Figure IV. 2.- Habitat complexity: path analysis results for the domain of records from rivers with at least 10 occurrences of freshwater pearl mussel, variance explained 23% .Path analysis could not be conducted on the alternate domain of 83 records because the correlation matrix was singular.

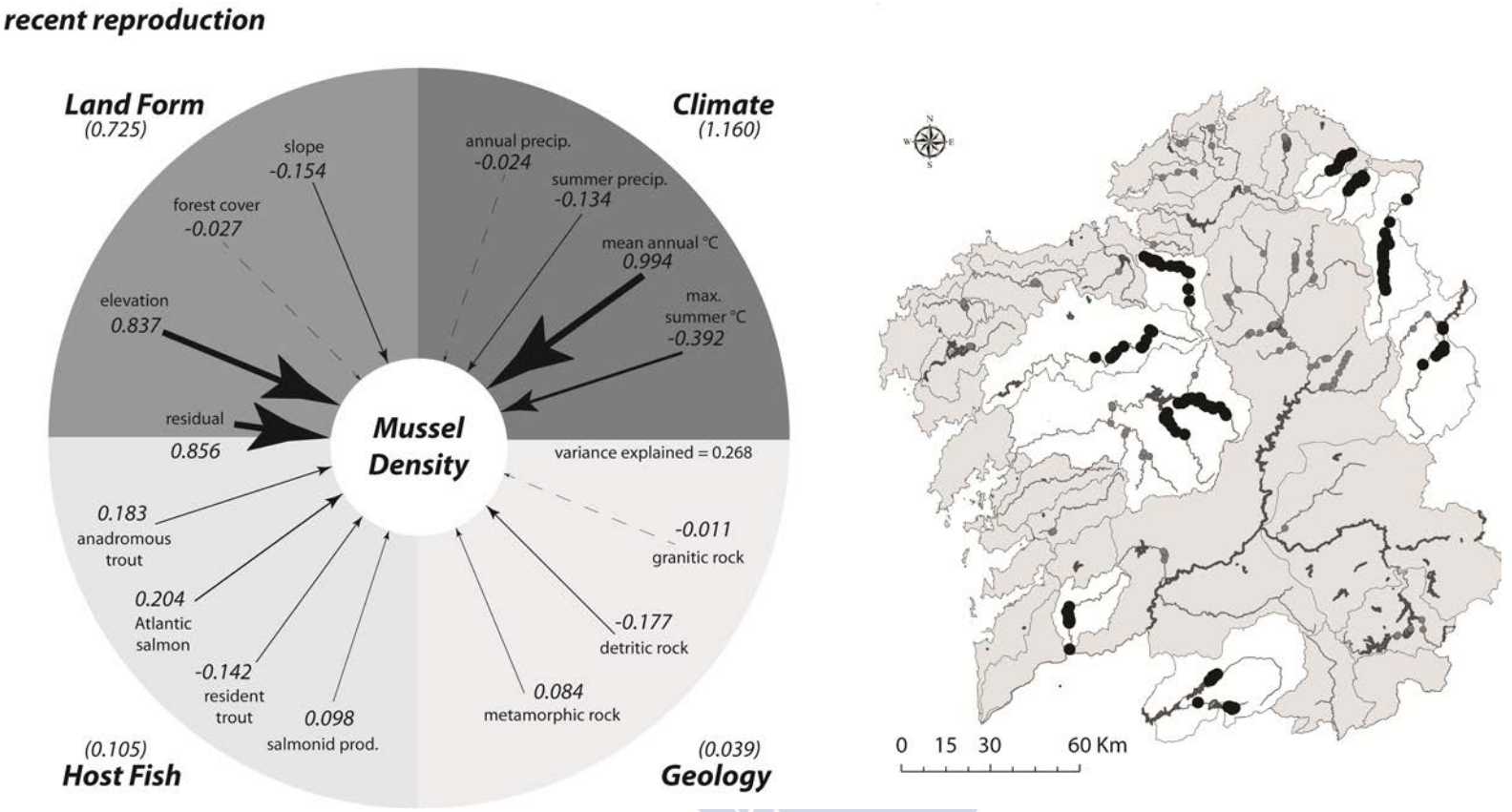

Figure IV.3.- Species complexity: Path analysis results for the domain of records with evidence of recent reproduction. For records from rivers with evidence of recent reproduction, the amount of variation explained was 26.8% whereas 26.1% of the variation was explained for records from rivers with no evidence of recent recruitment (Table IV.1).

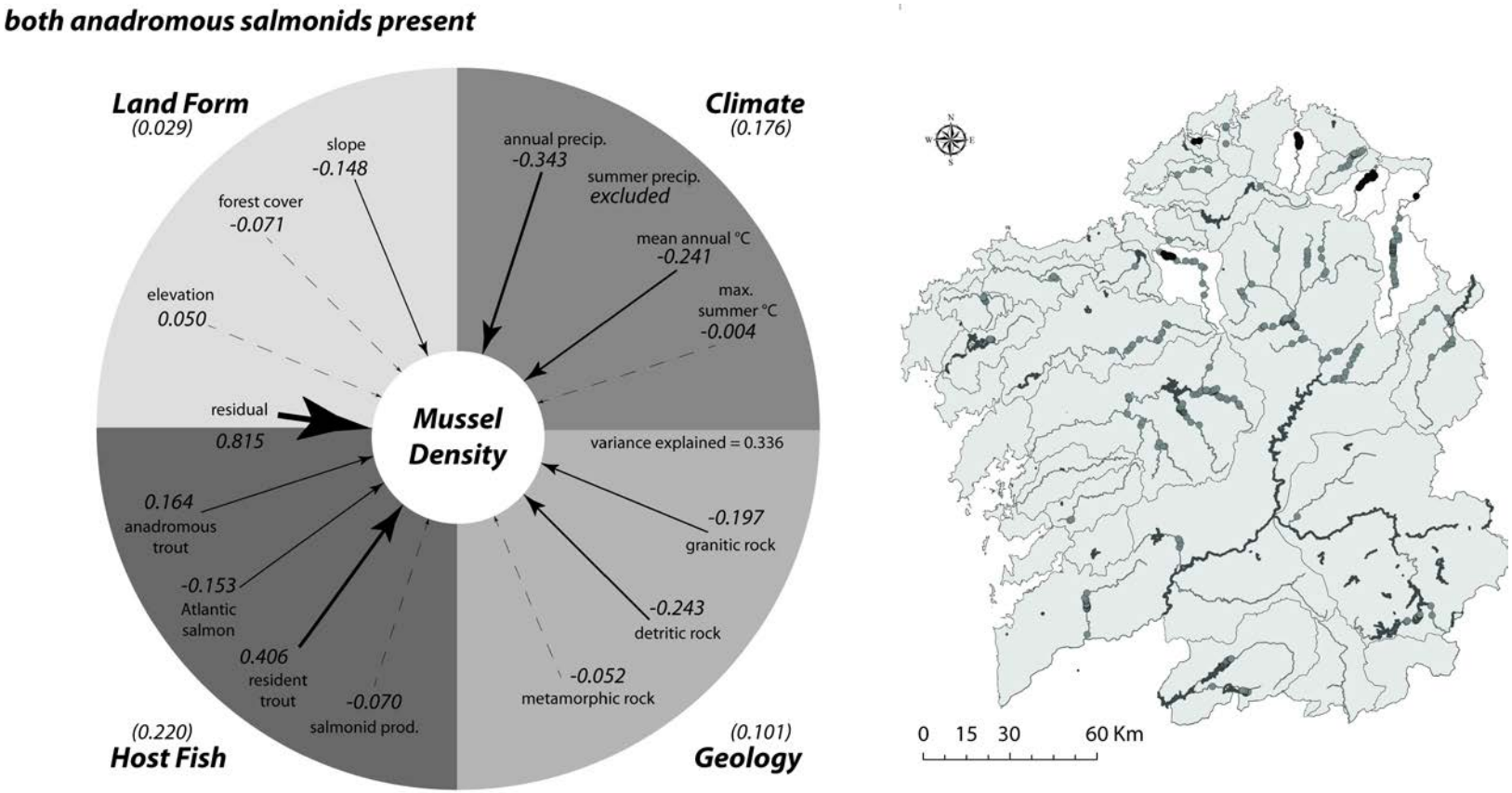

Figure IV.4.- Host fish complexity: Path analysis results for the domain of records where both anadromous salmonids were present. For records from rivers with presence of both anadromous salmonids, the amount of variation explained was 33.6% whereas 15% of the variation was explained for records with absence of anadromous salmonids. (Table IV.1).

Variable	Indirect	Total Effect
Excluding rivers with < 10 records *(path figure IV.2)*		
detrital rocks	-0.128	**-0.275**
metamorphic rocks	0.022	0.145
granitic rocks	-0.095	-0.064
elevation	**-0.645**	-0.035
forest cover	0.103	0.083
slope	**0.248**	0.142
salmonid prod.	-0.035	0.089
anadromous trout	0.056	**0.176**
Atlantic salmon	-0.107	0.086
resident trout	0.094	-0.083
annual precip.	**-0.344**	**-0.285**
summer precip.	0.014	**-0.288**
mean annual °C	**-0.558**	0.060
max. summer °C	0.112	-0.132
Records in rivers with recent reproduction *(path figure IV.3)*		
detrital rocks	-0.049	**-0.227**
metamorphic rocks	0.193	**0.278**
granitic rocks	-0.081	-0.092
elevation	**-0.974**	-0.137
forest cover	**0.159**	0.132
slope	**0.362**	**0.209**
salmonid prod.	0.017	0.115
anadromous trout	0.062	**0.245**
Atlantic salmon	-0.090	0.115
resident trout	0.083	-0.059
annual precip.	**-0.248**	**-0.272**
summer precip.	**-0.168**	**-0.302**
mean annual °C	**-0.841**	**0.153**
max. summer °C	**0.223**	**-0.170**

Variable	Indirect	Total Effect
Records with no recent reproduction *(path figure not shown)*		
detrital rocks	**-0.212**	-0.094
metamorphic rocks	**-0.164**	**-0.204**
granitic rocks	-0.006	**0.293**
elevation	**-0.407**	**0.284**
forest cover	-0.123	**-0.160**
slope	0.024	-0.016
salmonid prod.	-0.090	-0.056
anadromous trout	**-0.156**	0.038
Atlantic salmon	-0.079	-0.005
resident trout	**-0.195**	-0.093
annual precip.	**-1.078**	**-0.238**
summer precip.	**0.665**	**-0.246**
mean annual °C	**-0.407**	**-0.201**
max. summer °C	**0.267**	0.020
Records with both anadromous salmonids present *(Figure IV.4)*		
detrital rocks	**0.191**	**-0.052**
metamorphic rocks	**0.275**	**0.223**
granitic rocks	0.011	**-0.186**
elevation	**-0.213**	**-0.163**
forest cover	-0.016	-0.087
slope	-0.013	**-0.161**
salmonid prod.	**0.317**	**0.247**
anadromous trout	**0.207**	**0.371**
Atlantic salmon	0.105	-0.048
resident trout	**-0.313**	0.093
annual precip.	-0.098	**-0.441**
summer precip.	---	---
mean annual °C	0.086	**-0.155**
max. summer °C	**0.219**	**0.215**

Table IV.1.- Indirect and total effects of environmental predictor variables for analyses shown in Figures IV.2, IV.3 and IV.4.

ANNEX V

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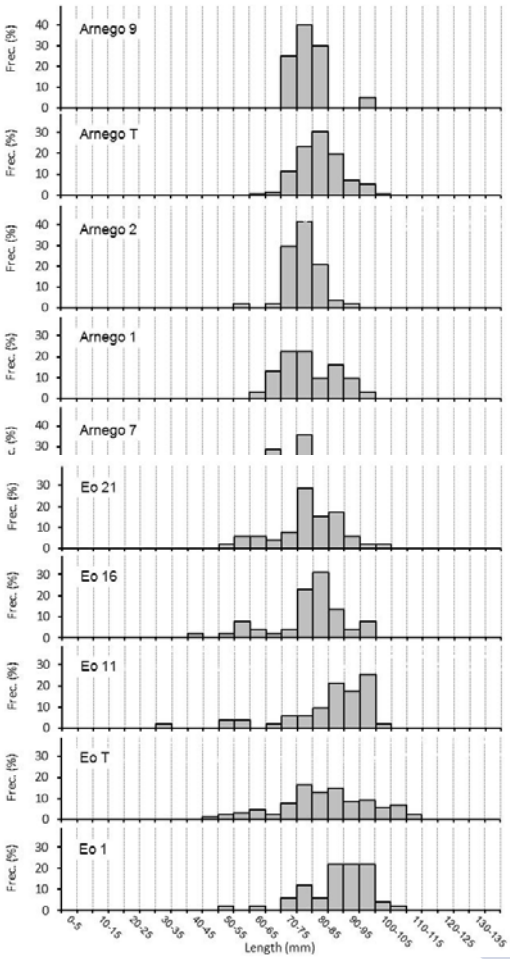
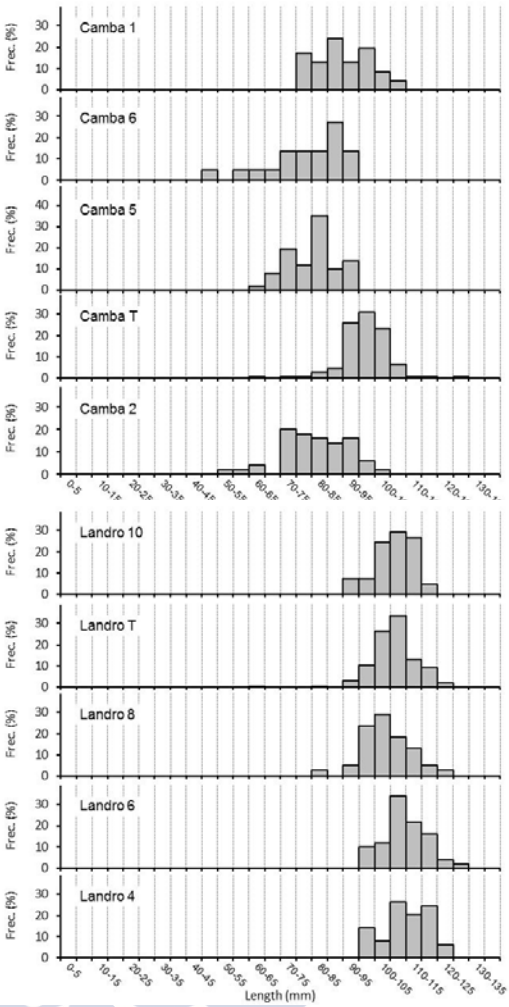
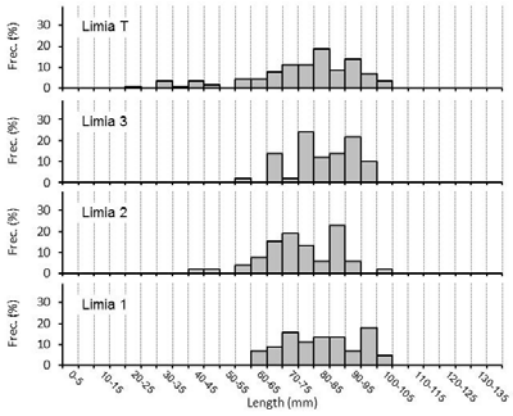
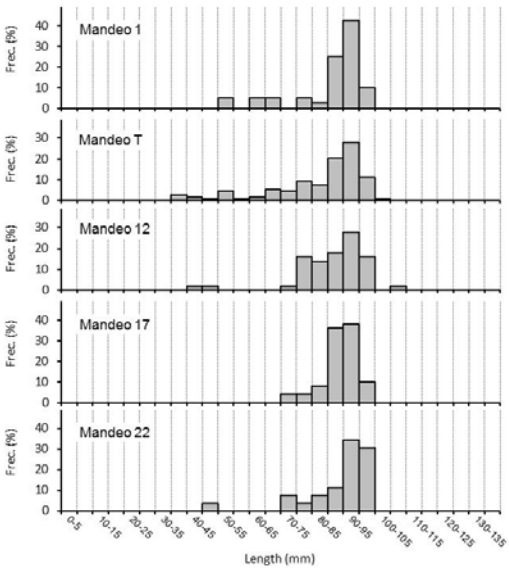
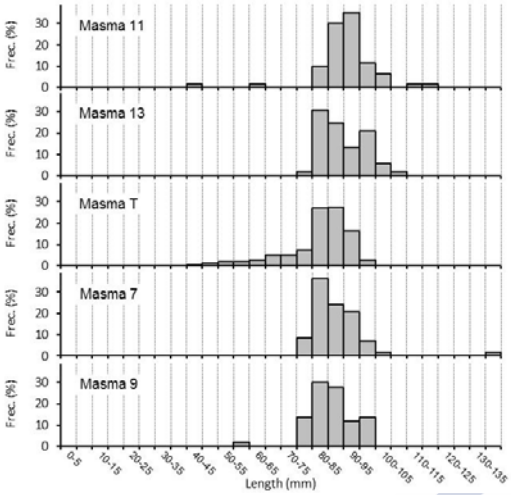
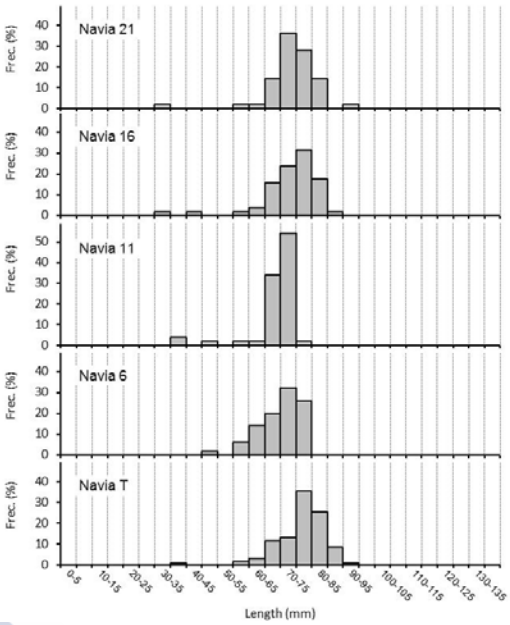

Arnego 9
Arnego T
Arnego 2
Arnego 1
Arnego 7
Eo 21
Eo 16
Eo 11
Eo T
Eo 1
Frec. (%)
Length (mm)

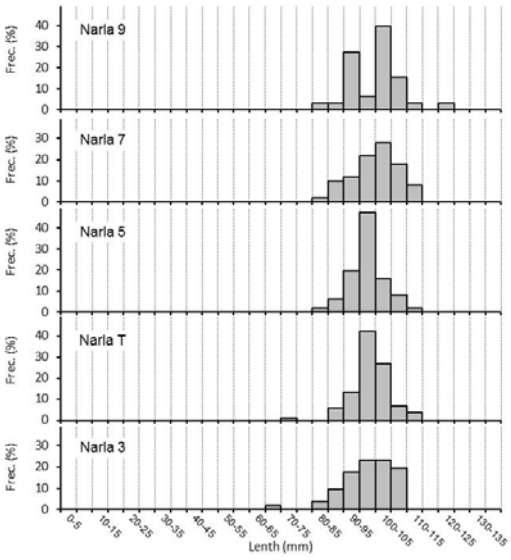

Camba 1
Camba 6
Camba 5
Camba T
Camba 2
Landro 10
Landro T
Landro 8
Landro 6
Landro 4
Frec. (%)
Length (mm)

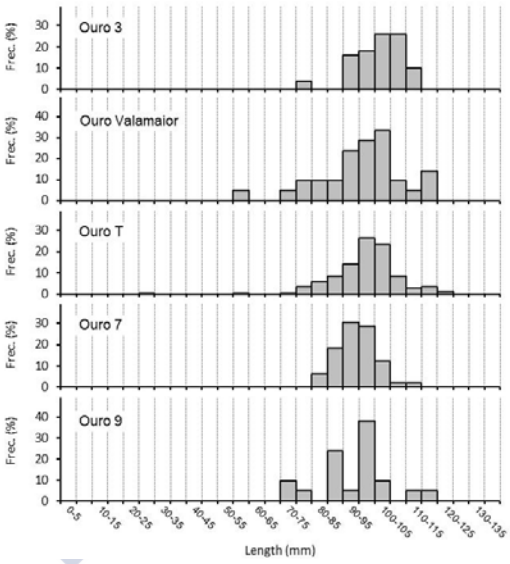

Limia T
Limia 3
Limia 2
Limia 1
Frec. (%)
Length (mm)

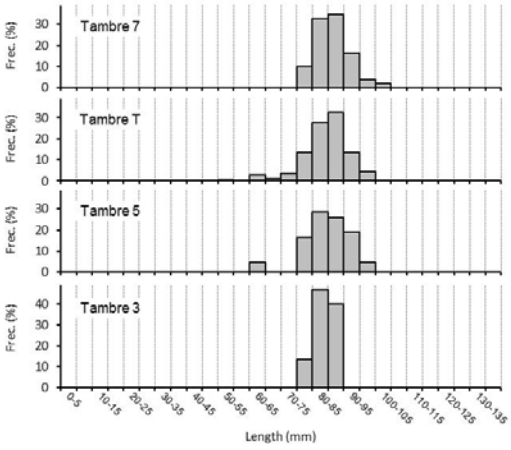

Mandeo 1
Mandeo T
Mandeo 12
Mandeo 17
Mandeo 22
Frec. (%)
Length (mm)

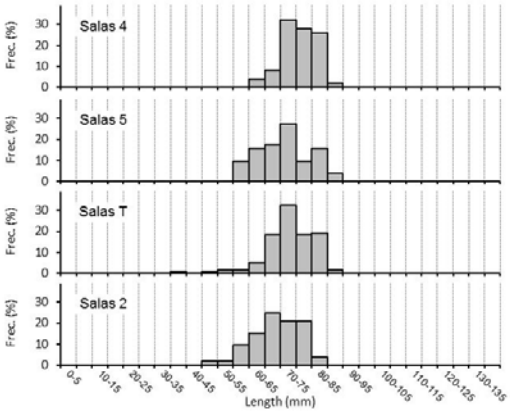

Masma 11
Masma 13
Masma T
Masma 7
Masma 9
Frec. (%)
Length (mm)


Navia 21
Navia 16
Navia 11
Navia 6
Navia T
Frec. (%)
Length (mm)


Narla 9
Narla 7
Narla 5
Narla T
Narla 3
Frec. (%)
Lenth (mm)


Ouro 3
Ouro Valamaior
Ouro T
Ouro 7
Ouro 9
Frec. (%)
Length (mm)


Tambre 7
Tambre T
Tambre 5
Tambre 3
Frec. (%)
Length (mm)


Salas 4
Salas 5
Salas T
Salas 2
Frec. (%)
Length (mm)

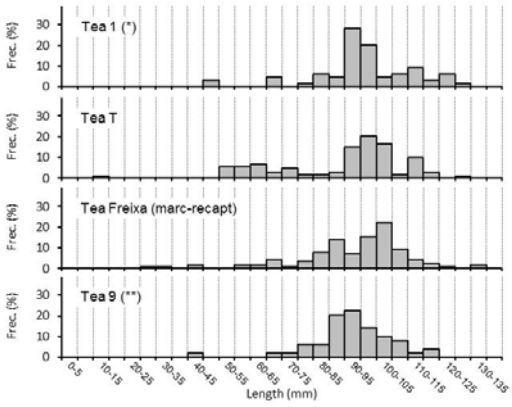


Figure V.1.- Length frequency distributions for different sites within 13 rivers studied. Groups of length frequency distributions are organized in alphabetical order by river name.